Wolbachia's Role In Classical Speciation Theory

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Abstract

Wolbachia are intracellular bacteria that commonly infect arthropod species. Since they often induce a cytoplasmic mating incompatibility (CI) in their hosts that acts as a postzygotic isolating mechanism between differently infected individuals of one species, *Wolbachia* have received attention as a potential promoter of arthropod speciation processes. Previous studies on speciation focused on either *Wolbachia*-induced or the classical nuclear-based postzygotic isolating mechanism. However, it should usually be the case that both co-occur. This thesis continues investigations on *Wolbachia*'s role in speciation by analyzing interactions of *Wolbachia*-induced CI and nuclear incompatibility (NI) caused by genetic differentiation. We will show that *Wolbachia* has strong impact on nuclear-based speciation processes. In particular, synergy effects can occur when both isolating mechanisms act simultaneously. Furthermore, we show that *Wolbachia* can influence speciation processes under more general conditions than previous studies on *Wolbachia*'s role in speciation suggested.

Since the actual role of *Wolbachia* in arthropod speciation will strongly depend on their abundance, we present a statistical meta-analysis of published data on *Wolbachia* infection frequencies. Due to the sampling methods applied in studies on *Wolbachia* infection frequencies, it is likely that current estimates of 20% infected species are underestimates. This is supported by our analysis and we show that more likely about two-thirds of species are infected.

Combining both results, this thesis provides strong evidence for *Wolbachia* being a very general factor in arthropod speciation processes.

Zusammenfassung

Wolbachien sind intrazeklluläre Bakterien die zahlreiche Arthropodenarten infizieren. Sie induzieren häufig eine zytoplasmatische Paarungsinkompatibilität die postzygotische Isolation zwischen unterschiedlich infizierten Individuen der gleichen Wirtsart verursacht, weswegen Wolbachien Beachtung als mögliche Katalysatoren von Artbildungsprozessen gefunden haben. Vorherige Arbeiten zur Artbildung untersuchten meist entweder Wolbachia-induzierte oder die klassischen, genetischen postzygotischen Isolationsmechanismen. Normalerweise sollte es aber der Fall sein dass beide Mechanism gleichzeitig auftreten. In dieser Arbeit führen wir Untersuchungen zur Rolle der Wolbachien in der Artbildung fort indem wir die Interaktionen von Wolbachia-induzierten und genetischen Inkompatibilitäten analysieren. Wir werden zeigen dass Wolbachien einen starken Einfluss auf genetisch-basierte Artbildungsprozesse haben. Insbesondere können sich die Mechanism bei gleichzeitigem Auftreten katalysieren. Außerdem werden wir zeigen dass Wolbachia Artbildungsprozesse unter allgemeineren Bedingungen beeinflussen kann als vorherige Studien suggerierten.
Da die Rolle der Wolbachien in der Artbildung stark von deren Verbreitung abhängt, werden wir desweiteren eine statistische Metaanalyse von bestehenden Daten zu Infektionsfrequenzen präsentieren. Aufgrund der Methoden der Datenerhebung ist es sehr wahrscheinlich, dass der wirkliche Anteil der infizierten Arten mit 20% deutlich unterschätzt wird. Unsere Analyse bestätigt dies und zeigt dass viel wahrscheinlicher circa zwei Drittel aller Arten infiziert sind.
Unsere Resultate der klassischen Artbildungstheorie kombiniert mit denen der statistischen Analyse zu Infektionsfrequenzen von Wolbachia implizieren dass Wolbachien als allgemeine Faktoren in der Evolution von Arthropoden anzusehen sind.

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Chapter 1

Overview

About 1.2 million described animal species are currently known to populate the earth. Doubtlessly, many more existing species are yet to be described scientifically and countless have gone extinct in recent history. Until 200 years ago, species were thought to be constant, unchangeable units that were created separately. Although Lamarck argued in 1809 that species might develop and evolve during their history of life, it was Darwin who first suggested that different species might have common ancestors and that, thus, species might emerge from other species. From the publication of *'On the Origin of Species'* (Darwin, 1859) until now, understanding this process of one species splitting into two distinct species has been one of the major challenges in biology and is still raising numerous questions. Darwin himself regarded natural selection as the driving force to adapt individuals to their particular environment. If subdivisions of one ancestral species populate different habitats, each group should change in response to the particular environment. By this process, subpopulations may diverge to eventually form two (or more) distinct species. Darwin, however, could not explain how such new attributes that are beneficial in a particular environment are passed on to the next generation and finally become established in the population.
A contemporary of Charles Darwin was Gregor Mendel. Mendel performed crossing experiments with pea plants and provided an explanation for the genetic inheritence of certain attributes with the publication of the *'Principles of Inheritance'* (Mendel, 1866). Apparently Darwin did not notice Mendel's work, and the fact that Mendel's experimental results provided strong support for Darwin's theory was only emphasized in the 1930's by Fisher, Haldane, Dobzhansky and other architects of the *Modern Synthesis*. One of its key ideas, that new 'Mendelian genes' evolve and natural selection acts to fix genes in particular populations, is still generally believed to be the basis for speciation events. Although scientific work up to now had shed

light on many evolutionary processes, major questions about how species diverge, what causes reproductive isolation between groups of a common former species and how hybrid dysfunctions such as sterility or inviability evolve cannot be fully explained. Usually, studies on the aforementioned topics focus on genetic factors. It is, however, possible that cytoplasmic non-genetic elements can influence speciation processes of their hosts. A symbiotic organism living in the cytoplasm of a certain host can only be transmitted to the next host generation via the egg of a female but not by the sperm of a male host. Therefore, to increase their own fitness, cytoplasmic elements have an interest in increasing the proportion of infected female hosts. Prominent representatives of such selfish elements are bacteria of the genus *Wolbachia*. *Wolbachia* infections are extremely common in insects, but other arthropod and nematode species harbor infections as well. *Wolbachia* infections can, for example, be responsible for reproductive isolation between sister species or for lethality of hybrid males. Since such patterns (when caused by genetic factors) are usually supposed to play an important role in speciation, there has been strong motivation for investigating *Wolbachia*'s role in speciation processes of their hosts. In particular, *Wolbachia* can induce a mating incompatibility in their hosts that avoids or reduces offspring production between infected males and uninfected females. This phenomenon was already observed by Laven (1959) who first suggested that cytoplasmic elements can have the potential to influence host speciation if they cause postzygotic isolation. Within the last two decades, numerous empirical and theoretical studies have supported this idea. Most of such studies and most studies on speciation processes have generally investigated the impact of either nuclear or cytoplasmic factors in speciation. However, it is very likely that both, nucleus-based and cytoplasmic factors occurred simultanuously.
In this work, we will continue the investigations on *Wolbachia*'s role in speciation. In contrast to former studies we will connect classical nucleus-based speciation theory with *Wolbachia*-related cytoplasmic factors and analyze their interactions.
In the following chapter 'Basics', we will introduce *Wolbachia* in general and in particular review studies on *Wolbachia*'s role in speciation. We further present important models and essential facts from classical speciation theory that will be applied within this work.
In chapters 3 and 4 we will investigate interactions of *Wolbachia* and genetic factors. Both are *per se* claimed (*Wolbachia*) or established (genetic factors) promotors of speciation processes. So far no study on speciation has considered both factors simultaneously, although it is very likely that both co-occur. We will show that co-occurence of both generally leads to reinforcement of the single factors. In particular, *Wolbachia* promote specia-

tion processes driven by genetic factors. The process of one species splitting into several distinct species is thus more likely to occur in *Wolbachia*-infected species.
Besides the potential to influence the evolution of their host species, *Wolbachia*'s actual role in speciation will crucially depend on their abundance. Among the 1.2 million described species, about one million, thus more than 80%, are insects of which a certain proportion is infected by *Wolbachia*. Current estimates suggest that around 20% of insect species harbor *Wolbachia* infections. However, due to the applied sampling methods, it is likely that a much higher percentage of species is infected. In chapter 5 we will present a statistical method to estimate infection frequencies based on available data. This is the first meta-analysis on *Wolbachia* infection frequencies and shows that 20% was an underestimate and that it is more likely that about two-thirds of species are infected. In particular, this implies that *Wolbachia* is present in at least every second described species.
Combining results from both parts, this work provides strong evidence for *Wolbachia* being an important and general factor in arthropod speciation processes. This is because we show that *Wolbachia*, embedded in a classical nucleus-based speciation theoretical framework, can promote speciation processes over a broad range of conditions. Splitting processes should thus occur more likely in *Wolbachia*-infected species. Since we also show that about two-thirds of species are infected, interactions between nuclear and *Wolbachia*-related factors should be a common process and *Wolbachia* should generally be considered as a promotor of speciation processes in arthropods.

Chapter 2

Basics

2.1 *Wolbachia*

2.1.1 History

The first detection of *Wolbachia* dates back to 1924, when Hertig and Wolbach (1924) found intracellular bacteria in the ovaries of the mosquito *Culex pipiens*. These bacteria were named *Wolbachia pipientis* later by Hertig (1936) in honor of his departed collaborator Wolbach. Besides the detection and classification of the bacteria as Rickettsia, no further investigations on the nature of the bacteria or possible interactions between hosts and endosymbionts had been undertaken. That a *Wolbachia* infection can be connected to certain abnormalities in the reproduction of *Wolbachia*'s hosts was stated for the first time in the mid 1970's. But already 20 years after the detection of *Wolbachia*, one of their modes of manipulating host reproduction was observed by Laven (1951). He performed crossing experiments between *Culex* mosquitos from Hamburg and Oggelshausen in the south of Germany. He found that males from Hamburg produced no or very few offspring with females from Oggelshausen, while the reciprocal cross between females from Hamburg and males from Oggelshausen produced normal numbers of viable offspring. Also, mating partners from the same locality did not show any reduction of reproductive sucess when mating with each other. Usually, this sort of hybrid breakdown was thought to be caused by genetic factors, but Laven stated that this incompatibility is due to maternally transmitted cytoplasmic factors and called it cytoplasmic incompatibility (CI). Furthermore, Laven (1959) pointed out that CI can be considered a potential mechanism in speciation. It was generally thought that speciation is initiated by such hybrid incompatibilities, but these were supposed to underlie genetic differences between interbreeding groups. However, when

cytoplasmic incompatibilities can build up isolating barriers just like genetic incompatibilities, why shouldn't there be an influence on speciation processes as well? That there exists a connection between *Wolbachia* and this cytoplasmic mating incompatibility was proposed another 20 years later by Yen and Barr (1971). In subsequent crossing experiments, Yen and Barr (1973) set the foundations for further research on *Wolbachia*. They found that mosquitos could be cured from infection by tetracycline treatment. Based on this, they could show that the incompatible crossing type occurs exclusively between infected males and uninfected females. Tetracycline-treated males, cured from infection, produced normal number of offspring with uninfected females. On the other hand, infected females were compatible with both, infected and uninfected males. That this mating incompatibility is not restricted to *Culex* mosquitoes but also found in other insects has attracted much interest. Molecular methods like polymerase chain reaction (PCR) nowadays provide a cheap and easy technique to test insect species for *Wolbachia* infections. Werren et al. (1995a) showed that *Wolbachia* were found in at least 16% of neotropical insect species, covering all major insect groups including Coleoptera, Diptera, Hymenoptera and Lepidoptera. This and further so called *Wolbachia* screenings confirmed that *Wolbachia* are extremely common on the one hand and distributed throughout all insect groups on the other hand. Besides CI, further *Wolbachia*-induced mechanisms have been reported (see section 2.1.4). Stouthamer et al. (1990) found endosymbionts to cause parthenogenesis in the wasp *Trichogramma*, manipulating infected virgin females to produced all female offspring. Rigaud et al. (1991) found infectious agents in wood lice, *Armadillidium vulgare*, converting genotypic males into functional females. These are able to produce eggs and to reproduce with non-infected, 'real' males. The fourth known mechanism is male-killing (MK). MK-*Wolbachia* have been found in several insect groups (Hurst et al., 1997) and cause a significant sex-ratio distortion by killing nearly all male embryos.
Due to their abbundance and the various ways they can manipulate the reproductive system of their hosts, *Wolbachia* became an important research subject. Nowadays, *Wolbachia* are thought to play a role in arthropod speciation and may have applications in pest control.

2.1.2 Phylogeny

Upon the first detection and description of *Wolbachia* in *Culex pipiens* (Hertig and Wolbach, 1924, Hertig, 1936), several other arthropod species were reported to harbor similar endosymbionts and to show equivalent mating incompatibilities. But since it has been impossible to culture bacteria outside

their hosts, there have been difficulties in determining the relationships between the symbionts of different host species. Within the last decades, the analysis of 16S rRNA molecules has become an accepted method to classify bacteria phylogenetically. To determine the relationship between different bacteria species, the amount of concurrent 16S rRNA sequences is measured. In general, two groups are defined to belong to the same species, if their sequences differ in less than 2%. However, it should be noted there are no established rules regulating the phylogenetic classification of microbes yet. O'Neill et al. (1992) sequenced 16S rRNA molecules of *W. pipientis* assigning them to the group of *α-proteobacteria*, with sister species *Ehrlichia*, *Anaplasma* and *Neorickettsia*. In the same study, 16S rRNA of six other intracellular bacteria from insect hosts was sequenced. It turned out that all of them formed a monophyletic group with *W. pipientis*. Meanwhile, numerous different *Wolbachia* strains have been found. Because 16S rRNA is considered to be a highly conservative, slowly evolving molecule, faster evolving genes needed to be considered to investigate relationships within the *Wolbachia* clade more accurately.

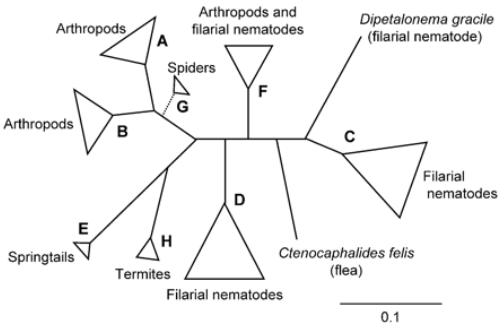

Figure 2.1: Diagram of *W. pipientis* phylogeny based on studies of $ftsZ$, $groEl$, $gltA$ and $dnaA$ genes (figure taken from Lo et al. (2007)).

The current division of different *Wolbachia* strains in so called supergroups A-H is based on the sequencing of different, single genes (Fig. 2.1). However, it was argued that analysis of single genes might be insufficient due to high recombination rates within supergroups. To obtain more reliable classifications, a multilocus sequence typing (MLST) was proposed by Paraskevopoulos et al. (2006) but such uniform procedure has not been established yet. So far, eight *Wolbachia* supergroups have been determined. Some divergent lineages (*Wolbachia* in filarial nematodes and fleas) have not been designated yet. Due to growing interest in *Wolbachia* research and the probable detection of further strains in further host species the number of supergroups will likely increase in the near future.

2.1.3 Biology of *Wolbachia*

Wolbachia are gram-negative eubacteria and occur either in rodlike ($0.5 - 1.3\mu m$ in length) or coccoid form ($0.25 - 0.5\mu m$ in diameter) but also in larger

forms ($1 - 1.8\mu m$ in diameter) containing some of the smaller forms Hertig (1936). *Wolbachia* are surrounded by three membrans. The innermost is the bacteria's plasma membrane enclosed by a bacterial cell wall. The outer membrane is of host origin and it is speculated that the host can control endosymbionts via these membrans.
The maternal transmission via the cytoplasm of the egg, i.e. how bacteria successfully enter hosts' germ cells, has not been fully understood yet. It was observed that *Wolbachia* are evenly distributed within female germ lines, but concentrate in the future oocyte during oogenesis. Once the oocyte is built, *Wolbachia* again disperse throughout the egg. There is evidence that *Wolbachia* utilize hosts microtubule cytoskeleton to localize in the particular cell parts (Ferree et al., 2005). There is further evidence that *Wolbachia* are capable to move from outside the reproductive tissues into the female germ line. Recently, Frydman et al. (2006) have shown that *Wolbachia* can cross different tissues to reach the germ line when injected into *Drosophila melanogaster*. This can have important implications for the horizontal transfer of *Wolbachia* (see section 2.1.6) across different host species. *Wolbachia* do not need to be exclusively found in the reproductive tissues of their hosts. In some insect species, also somatic tissue like muscles (Dobson et al., 1999) or nervous tissue (Rigaud et al., 1991) can be infected with bacteria, where the latter suggests a possible influence of bacteria on the hosts behavior.

2.1.4 Reproductive Parasitism

Wolbachia are predominantly found in the reproductive tissues of their arthropod hosts. They are usually transmitted vertically to the next host generation over the cytoplasm of the egg (but horizontal transmission between species has occurred as well (see section 2.1.6)). Infected females transmit the infection to their offspring via the egg, whereas males cannot transmit bacteria by sperm. If a bacterium is transmitted to male progeny, it is *buried alive* since it has no possiblity to directly infect its host's offspring. From *Wolbachia*'s perspective, males are an evolutionary dead end, but infected females are the guarantors for the maintenance of bacteria in the host population. Apparently, bacteria benefit from an increased proportion of infected females in the host population. In fact, *Wolbachia* have the ability to alter the reproduction of their hosts to their own advantage. So far, there are four known such strategies that increase the percentage of infected females in the host population: cytoplasmic incompatibility (CI), male-killing (MK), parthenogenesis and feminizing. We introduce the four of them below and point out possible effects on the evolutionary processes of *Wolbachia*'s hosts. Since this work will focus on the impact of CI on arthropod speciation, CI

and its impact on speciation will be discussed again and in more detail in section 2.3.

Cytoplasmic Incompatibility (CI)

Cytoplasmic incompatibility is the most common and probably the most intensively studied mechanism induced by *Wolbachia* in their arthropod host. CI was first observed by Laven (1951) in crossing experiments with mosquitos *Culex pipiens*. Among others, he crossed mosquitoes from Hamburg with individuals of the same species from Oggelshausen. A mating incompatibility was detected between males from Hamburg and females from Oggelshausen, with the two being unable to produce the normal number of progeny when mating with each other. In contrast, all other matings including the reciprocal cross between males from Oggelshausen and females from Hamburg resulted in full number of progeny. Laven stated that this incompatibility has cytoplasmic (non-genetic) causes. The responsible cytoplasmic factor is present in the Hamburg population and transmitted to offspring by mothers only, but not present in individuals from Oggelshausen. That this cytoplasmic incompatibility is induced by the bacteria *Wolbachia* was later shown by Yen and Barr (1971). Since then, numerous cases of *Wolbachia*-induced CI in insects but also other arthropod species have been reported. It was found, amongst others, in Arachnida (Breeuwer, 1997), Crustacea (Moret et al., 2001) and insects belonging to Coleoptera (Wade and Stevens, 1985), Diptera (Hoffmann et al., 1986) and Lepidoptera (Hiroki et al., 2004).

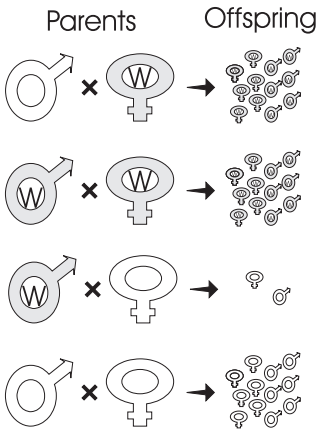

Figure 2.2: Cytoplasmic incompatibility. Unidirectional CI occurs between infected males and uninfected females and results in reduced offspring production. Other matings are compatible and produce the full number of progeny.

CI has been described as a modification-rescue(mod-res)-system (Werren, 1997). Occurring in males, *Wolbachia* cause a certain modification of sperm. As a result, sperm cannot fertilize an egg unless the same *Wolbachia* strain is present in the egg and rescues the embryo. In contrast, if the egg is uninfected or harbors a different strain, the rescue process is not initiated. This usually results in zygotic death. By this mechanism, *Wolbachia* captured in males ensure that their host can only reproduce with females harboring *Wolbachia* with the same mod-res-system that transmit this *Wolbachia* strain to the common offspring. CI is typically encountered in two variations: uni-

and bidirectional. Unidirectional CI, involving one *Wolbachia* strain only, occurs when an infected male mates with an uninfected female. The result is a reduced number of offspring in these crosses, whereas the reciprocal cross is compatible. Bidirectional CI involves two *Wolbachia* strains of different mod-res-systems. Mating partners carrying different strains produce no offspring or less than mating partners harboring the same strain. The proportion of inviable offspring in incompatible matings (CI level) is diverse. It ranges from very weak CI in *Drosophila* (Hoffmann et al., 1994) to almost perfect CI in *Nasonia* (Breeuwer and Werren, 1990). The strength of CI can also depend on the hosts nuclear background or bacterial density within host tissues (Bordenstein and Werren, 1998) as well as on the age of the host (Bressac and Rousset, 1993).
The mechanisms of CI are still unclear. Cytological studies have shown that the parental chromosome is lost during the first mitotic cell division, due to delayed chromosome condensation (for a review see Tram et al. (2003)). In diploid species, this typically results in zygotic death. In haplodiploid species, CI can also prevent offspring production but can lead to all-male offspring as well (Tram et al., 2006). In haplodiploid wasps *Nasonia*, both types of CI were observed. In *Nasonia vitripennis* for example, CI leads to paternal chromosome loss in fertilized eggs, but the emerging haploid egg then develops into a male whereas in *N. giraulti* and *N. longicornis* CI results in the death of embryos.

Male-Killing (MK)

Male-killing (MK) endosymbionts can dramatically distort sex-ratios of their host populations. Male killers kill their male hosts at the embryonal stage but do not interfere with the development of their female hosts. Killing male offspring is beneficial for *Wolbachia* presumed that siblings compete for food or cannibalism takes place within broods (Hurst et al., 1997). In the first case, as there is less competition within infected broods, infected daughters gain a fitness advantage over uninfected daughters that have to rival their uninfected living brothers. In the second case, dead male embryos provide an additional meal to their sisters if siblings feed on unhatched eggs. MK-*Wolbachia* have been found in butterflies (Hurst et al., 1999, Dyson et al., 2002), *Drosophila* (Dyer and Jaenike, 2004, Hurst et al., 2000), beetles (Fialfo and Stevens, 2000, Hurst et al., 1999) and quite recently in a non-insect species of pseudoscorpions (Arachnida) (Zeh et al., 2005). Moreover, male-killing seems to be a more general pattern within reproductive parasitism. Besides *Wolbachia*, there are further endosymbionts as *Spiroplasma* or *Rickettsia* that kill male progeny of their hosts (see Hurst and Jiggins (2000) for

an overview). This diversity of male-killers has given evidence for this trait having evolved more easily than other interventions in the hosts' reproductive system. For instance, parthenogenesis-induction has been found only in association with *Wolbachia*, but not with other bacteria.

Usually, the percentage of infected individuals in natural populations varies from 5-50% (Hurst and Jiggins, 2000), but can also reach fixation. The actual infection densities depend on transmission rates, i.e. the proportion of infected progeny produced by an infected mother or the efficiency of the male killer, i.e. the percentage of infected sons that die. For MK-*Wolbachia*, various infection frequencies have been observed. Hurst et al. (1999) found a low prevalence of 5% in the butterfly *Adalia bipunctata*. On the other hand, transmission rates and the degree to which infected sons are killed can be nearly perfect so that the sex-ratio is distorted dramatically. An infected population of the butterfly *Acraea encedana* showed a prevalence of *Wolbachia* of 95% among females and only 6% of collected individuals were males (Jiggins et al., 2000). A similar high prevalence of *Wolbachia* ranging from 61-95% was observed in the butterfly *Acraea encedon* (Jiggins et al., 1998). Paradoxically, females - whether infected or not - need males to reproduce and if transmission rate is nearly perfect, *Wolbachia* run the risk to exterminate their host population and themselves. An extraordinary case was reported from a population of *Hypolimnas bolina* on Fiji, where Simmonds (1923) did not succeed to find any males. Some decades later, Clarke et al. (1983) showed that this extreme sex-ratio distortion has persisted for at least 150 host generations. Presuming that the vertical transmission of *Wolbachia* can in fact reach 100%, this poses the problem of how the MK-infection and thus the whole host population can survive. Theoretical studies (Randerson et al., 2000) imply that this strong sex-ratio distortion entails the evolution of mate choice of males, preferring uninfected females to produce progeny with, so that a sex-role reversal appears. It was also shown that this choosiness of males enables a long-term maintenace of a male-killing bacterium with almost perfect transmission rate in a population.

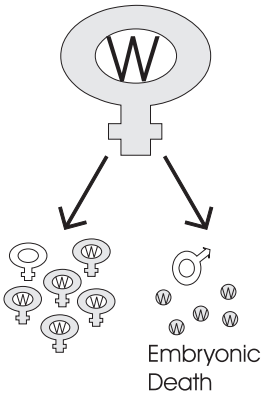

Figure 2.3: Male Killer. MK-*Wolbachia* cause the death of their infected male hosts, whereas daughters or uninfected males (transmission usually less than 100%) remain viable.

Parthenogenesis

Parthenogenesis-inducing *Wolbachia* have been reported to infect more than 40 insect species. Remarkably, all these species belong to the Hymenoptera. This is probably due to the cytological mechanism (Stouthamer and Kazmer, 1994) inducing thelytoky that is only applicable under haplodiploid sex determination. In species with haplodiploid sex determination system males are haploid and females diploid. Mothers can determine the sex of their progeny by either fertilizing eggs (→ daughters) or leaving eggs unfertilized (→ sons). In the wasp *Trichogramma*, *Wolbachia* induce parthenogenesis (Stouthamer and Kazmer, 1994). If infected females intend to produce haploid males from unfertilized eggs, *Wolbachia* disrupt the first mitotic division so that a diploid nucleus is maintained. The diploid cell then develops into a daughter. If eggs are fertilized, the development proceeds as usual without any manipulations by *Wolbachia*. Thus, independent of the intended sex-ratio by the mother, *Wolbachia* succeed to manipulate the reproduction of their host in a manner that only infected females are produced. Most notably, there is no need for males anymore. On the contrary, MK-populations would become extinct if *Wolbachia* was transmitted to all male offspring and killed all of them. Parthenogenesis-inducing *Wolbachia* can, nevertheless, cause fecundity reductions in infected females as well (Stouthamer and Luck, 1993). So do infected females usually produce less progeny than uninfected females. Further, the transmission of *Wolbachia* might be incomplete, i.e. *Wolbachia* do not infect every descendant of an infected mother. In these cases, infection polymorphisms occur, that is the coexistence of infected and uninfected (males and females) individuals.

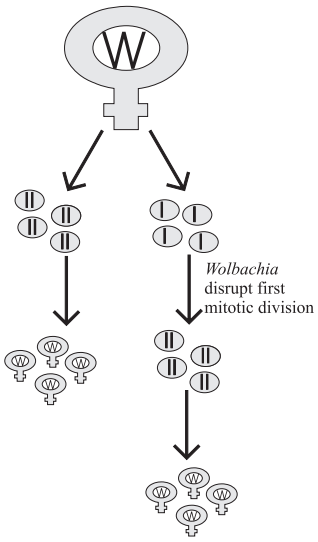

Figure 2.4: Parthenogenesis. *Wolbachia* disrupt the first mitotic cell division. A diploid nucleus is maintained resulting in the production of diploid females from actually haploid eggs.

Zchori-Fein et al. (1992) reported perfect parthenogenesis-induction in the parasitric wasp *Encarsia formosa*. Although infected females produced less progeny than tetracycline cured females, the transmission rate seemed to be perfect and males were not produced at all. *E. formosa* have first been described in 1924 and since then all populations have been classified as asexual. Antibiotic curing of infected females led to the production of sons. Those were able to produce sperm and occasionally mated with females, but insemination did not occur. This could be an example of perfect reproductive

parasitism. Due to the maternal transmission, males are *useless* (Stouthamer et al., 1999) from the endosymbionts point of view. In *E. formosa*, *Wolbachia* might have succeeded to totally eliminate males and to render their host species asexual.

Feminizing

Feminizing *Wolbachia* were first reported to infect the woodlouse *Armadillidium vulgare* (Rigaud et al., 1991) and cause a highly female-biased sex-ratio. They do so by converting genotypic males into functional females, which are able to mate with real (genotypic) males and produce offspring. Woodlice have a ZW sex determination system: males are homogametic (ZZ) whereas females are heterogametic (ZW). *Wolbachia* create an intersex (ZZ+*Wolbachia*) that has a male genotype but behaves sexually as a female. As a result, *Wolbachia*-infected populations seem to be highly female-biased, but actually the female sex chromosome W is rare since false females (ZZ+*Wolbachia*) lack the W chromosome and thus produce genotypic male offspring only.

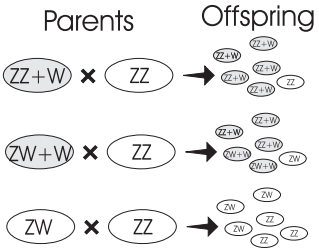

Figure 2.5: Feminizing-*Wolbachia*. Illustrated are possible crossings in an infected population. Genotypic males can, if infected, reproduce with real, uninfected males.

In general, maternal transmission of *Wolbachia* is imperfect, i.e. infected females (ZW) produce a small proportion of uninfected male offspring. But still, the sex-ratio is usually extremely (false) female-biased. One would usually expect that selection pressure acts to favor the production of sons in order to reestablish a Fisherian sex ratio of 1:1. In *A. vulgare*, Rigaud and Juchault (1993) detected a masculizing gene, surpressing the female determinant on the W chromosome and converting the genetic female to a functional male. Also, perfect maternal transmission of feminizing *Wolbachia* has been observed to have crucial consequences on the sex determination system of the host. In some populations of *A. vulgare*, the W chromosome has disappeared (Rigaud and Juchault, 1993) so that all individuals are genetic males, either infected and thus functional females or uninfected *real males*. Feminizing *Wolbachia* are widespread among isopod crustaceans (Bouchon et al., 1998) and it has been speculated that they are restricted to isopods due to female heterogamety. Kageyama et al. (2002) were the first to detect a feminizing *Wolbachia* in an insect species, *Ostrinia furnacalis* (Lepidoptera). Thus, feminizers are apparently not re-

stricted to infect isopods only. However, Lepidoptera have the same sex determination system as isopods with heterogametic females which led to the assumption that feminizers could evolve more easily in species with female heterogamety. In 2006, however, Negri et al. (2006) found feminizing *Wolbachia* in leafhoppers (Hemiptera), an insect species with XX (females)/X0(males) sex determination.

2.1.5 Distribution of *Wolbachia*

Wolbachia in Arthropods

Wolbachia belong to the most common endosymbionts of arthropods. Molecular techniques such as PCR facilitated the detection of *Wolbachia* in arthropods and many surveys on the distribution of *Wolbachia* have been published within the last decade (O'Neill et al., 1992, Werren et al., 1995a). Most of these studies consistently estimated the incidence of *Wolbachia*, i.e. the proportion of infected species, to be around 20%. Jeyaprakash and Hoy (2000) used a more sensitive techniques and obtained an infection frequency of 76%, but it is argued that their method is prone to produce false positives. Among insects, *Wolbachia* have been shown to infect all major orders including Coleoptera, Lepidoptera, Diptera, Hymenoptera, Hemiptera/Homoptera and Orthoptera (Werren et al., 1995a). As non-insect arthropods, Crustaceans (Rousset et al., 1992, Bouchon et al., 1998) and Arachnids (Breeuwer and Jacobs, 1996) were found to harbor *Wolbachia*. A major problem of these studies is that of most species only one individual was collected. If this was infected, the species was rightly classified as infected. An uninfected individual, however, resulted in the classification of this species to be uninfected. It is obvious that estimates obtained by such methods underestimate the number of infected species, as infections do not necessarily need to occur at 100% prevalence. This issue has been remarked in many of the surveys and has been, in part, tried to be compenstaed by extensive sampling. In chapter 5 this problem will be discussed in detail. Current data will be analyzed with statistical methods. In this meta-analysis, it can be estimated which proportion of negative tested species were falsely classified as uninfected. The analysis confirms that frequency of *Wolbachia* has been underestimated and implies that two thirds of tested species harbor *Wolbachia*.

Wolbachia in Nematodes

Intracellular bacteria in filarial nematodes were first detected in the 1970's in *Dirofilaria immitis* and *Brugia pahangi* (McLaren et al., 1975), in *Brugia*

malayi (Kozek, 1977) and *Onchocerca volvulus* (Kozek and Marroquin, 1977). McLaren et al. (1975) further speculated that these bacteria might be related to *Wolbachia*, intracellular bacteria that were already known to infect arthropods. Although it was proposed that these bacteria might contribute to the pathogenesis of filarial disease and provide a novel target for chemotherapy, *Wolbachia*-nematode symbioses did not attract much attention. When Sironi et al. (1995), using new molecular techniques (PCR, 16S rRNA), detected close relatives of *Wolbachia* in a dog heartworm, more interest was drawn to this research area. Meanwhile, *Wolbachia* have been detected in the majority of nematode species (Taylor and Hoerauf, 1999).
While in arthropods *Wolbachia* are known to alter the reproduction of their host in various ways (section 2.1.4), no such modifications have been observed in filarial nematodes. Moreover, *Wolbachia* infections in nematodes are rather mutualistic. Arthropods can be cured from a *Wolbachia*-infection by antibiotic curing without any negative effects on hosts' viability or fertility. In contrast, curing a nematode from infection can result in sterility (Hoerauf et al., 2000) or even in the death of the nematode host (Taylor et al., 2005). Important implications for curing filarial deseases in humans arose (Hoerauf et al., 2000, Taylor et al., 2005). *O. volvulus* causing river blindness and *Wuchereria bancrofti* causing elephantiasis both infect millions of people predominantly in Africa, South America and other (sub-)tropical regions. Both parasites are themselves parasitized by *Wolbachia*. Treating the (human) patient with antibiotica can cure his nematode parasite from *Wolbachia*. This usually has the effect that the nematode either dies or is unable to produce offspring. *Wolbachia* might thus play an important role as a target for chemotherapy and help to cure numerous people from filarial deseases.

2.1.6 Vertical and Horizontal Transmission of *Wolbachia*

The vertical, i.e. maternal transmission is the main way for *Wolbachia* to persist and spread in host populations. Combined with the *Wolbachia*-induced alterations of host reproduction, intra-species maternal transmission guarantees *Wolbachia*'s survival over numerous host generations. It is furthermore possible that *Wolbachia* is horizontally transmitted, from one species to another. Evidence comes from detecting closely related *Wolbachia* strains in different arthropod species that diverged long before *Wolbachia* strains diverged. Such a case was reported by Werren et al. (1995b) proposing that horizontal transfer can occur between arthropod parasite-host-associations.

Parasitic wasps *Nasonia* sting and lay eggs into the pupae of certain host fly species. While *N. vitripennis* can parasitize numerous fly species, *N. giraulti* and *N. longicornis* are specialized on protocalliphorid flies. Interestingly, *Wolbachia* strains found in both *Nasonia* species and in their fly host are closely related. In contrast, *Wolbachia* strains found in the generalist *N. vitripennis* do not show close relation to that in the protocalliphorid fly. A further example might come from other parasitric wasps *Trichogramma*. Remarkably, the different parthenogenesis-inducing *Wolbachia* strains in *Trichogramma* species are closely related and form a monophyletic group. Schilthuizen and Stouthamer (1997) have shown that *Wolbachia* strains must have diverged before the *Trichogramma* hosts implying that horizontal transmission is likely to have taken place and is supposed to have occurred when different *Trichogramma* species shared a common host.
Horizontal transmission has not been fully understood yet. Under laboratory conditions, one *Wolbachia* strain can be transferred to a different host species through microinjection. How horizontal transmission occurs under natural conditions remains to be studied. One study by Rigaud and Juchault (1995) showed that transmission is possible via blood-to-blood contact, but this is probably only one of many possibilities.
In nematodes, there is no evidence for intertaxon transmission. Within supergroups C and D, phylogeny of *Wolbachia* is congruent with the phylogeny of their nematode hosts (Bandi et al., 1998). Furthermore, data collected so far suggest that single nematode species are infected by one *Wolbachia* strain only, independent of their geographical distribution (Taylor and Hoerauf, 1999). In contrast, double infections or infection polymorphisms are known in some arthropod species. Regarding transmission of *Wolbachia* from arthropods to nematodes or vice versa, supergroup F is of special interest because this is the only group containing *Wolbachia* strains infecting both, arthropods and nematodes. A strain found in termites was also found in the filarial parasite *Mansonella ozzardi* (Lo et al., 2002), indicating that transmission from arthropod to nematode or vice versa is possible.

2.2 Speciation

What is a Species?

Understanding speciation processes, i.e. the splitting of one species into two, has been one of the major challenges in biology. The first arising question is, of course: What is a species? The most popular definition of species is certainly the biological species concept (BSC) by Mayr (1942):

> *Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups.*

This BSC, however, bears one crucial problem. What, if two groups can interbreed but emerging offspring does not reach adult stage or is sterile? In this case, the BSC does not provide a clear answer whether these groups should be considered distinct species or not. It is thus not surprising that new alternative formulations of species concepts arose, especially because current methods in genetical research provide new possibilities for the analysis and classification of organisms. Instead of the ability to reproduce, species can also be defined regarding genetic distance. Also evolutionary history can be consulted for the formulation of species concepts, and the condition that species do not reproduce with other species at all can be relaxed. So did Mallet (1995) by presenting an alternative species concept that is based on a definition by Darwin (1859) but updated in concordance with current knowledge about genetics.

> *A species is a [morphologically or genetically] distinguishable group of individuals that has few or no intermediates when in contact with other such clusters.*

Unfortunately, there is no general consensus on a particular concept among biologists. That the definition of 'species' is complex is obvious. Especially during the splitting process itself, determining when a new species has evolved or when the particular group should rather be called variety, subspecies etc. seems to be impossible.
In this work, we will consider very early processes in speciation and analyze models on the establishment of first isolating barriers (section 2.2.1) between groups of one ancestral species. These groups will be refered to as *populations*, *subpopulations*, *subgroups* etc. We will avoid using terms such as *species* or *subspecies* in order to obviate confusions due to their vague definition.

2.2.1 Isolating Barriers

The process of one species splitting into two or more distinct species requires the establishment of reproductive isolating barriers between groups of a common, ancestral species. Such isolating barriers are features of organisms that cause less reproductive success in intergroup matings and decrease gene exchange between groups. Gene exchange can still be existent, but must be reduced compared to intragroup matings. With the further progress and finally the completion of the speciation process, gene exchange will generally disappear.
Differents kinds of isolating barriers can evolve and these can be classified depending on when they act during reproduction:

1. ***Premating, prezygotic isolating barriers***
   Premating isolating barriers act before sperm is transferred to mating partners of another group. This can be caused by geographical isolation or females being choosy, i.e. females showing preferences in mate choice.

2. ***Postmating, prezygotic isolating barriers***
   Postmating, prezygotic isolating barriers allow mating of individuals of different groups but prevent the normal development of the arising zygote. Examples for such barriers are inappropriate copulation behavior of mating partners or gametes being unable to effect fertilization.

3. ***Postzygotic isolating barriers***
   Postzygoting isolating barriers allow matings between individuals of different groups and even the production of offspring. Hybrids, however, cannot produce offspring themselves because they either die before reaching adult stage or are sterile.

So far, premating isolating barriers have achieved more attention and are supposed to prevent gene flow more efficiently than postzygotic isolating barriers. However, different isolating mechanisms cannot be considered completely separated from each other. It has been shown theoretically that pre- and postzygotic isolation strengthen each other (Servedio and Saetre, 2003) or that postzygotic isolation selects for the evolution of mating preferences, called *'reinforcement'*. This follows intuitively. Females, recognizing that they have less reproductive success when mating with a certain type of males, choose those males as mating partners they produce more fit offspring with. These choosy females should spread within a population and a

prezygotic isolating mechanism would be established. As a result, different isolating mechanisms can depend on each other because one isolating barrier can provide the basis for a further one to evolve. Throughout this work we will focus on postzygotic isolating barriers. Therefore, the rest of this chapter predominantly deals with hybrid sterility or inviability but we will take up reinforcement later in this work (sections 2.2.5 and 2.3.2).

2.2.2 Genetics of Postzygotic Isolation

The evolution of hybrid sterility or lethality has been considered a paradox: How can natural selection allow the production of sterile or inviable organisms? This problem is easily formalized: Let genotypes aa and AA describe genotypes of two groups originating from a common ancestral population. The heterozygous hybrid genotype is aA and is supposed to be inviable or sterile. But how can one group evolve from the other? If a mutant allele A arose in an aa-population, genotype aA would appear as the first foreign genotype. Because these individuals are supposed to be sterile or inviable, natural selection should not allow the spread of A and thus an AA-population should not be established. To draw a picture of such scenario, one can assume that groups aa and AA sit on different adaptive peaks in a so called fitness landscape and are separated by an adaptive valley (aA). The question is how one of them passes that adaptive valley when natural selection is preventing this development. Of course, fixation of mutant alleles does not need to be promoted by natural selection. Genetic drift can also lead to the fixation of neutral or even deleterious alleles (see section 2.2.3).
Dobzhansky (1937)and Muller (1942) developed a model approach that allows the evolution of hybrid incompatibilities without the overcoming of adaptive valleys. The key idea is that incompatibilities are not caused by different alleles at one locus, but by different alleles being located at least at two different loci. This Dobzhansky-Muller model is described in detail in the next section because is will play a fundamental role in both chapters 3 and 4. Before, we want to remark that it is sometimes claimed to correctly call it the Bateson-Dobzhansky-Muller model (Orr, 1996). This is because Bateson (1909) provided the two-loci solution around thirty years earlier but his work was not recognized by other researchers. Probably not knowing that Bateson had had the same idea, Dobzhansky (1937) and Muller (1942) presented their approaches independently of each other. We want to remark that Bateson (1909) indeed formulated the (genetical) key idea of the model. He did not, however, incorporate (population genetical) geographical factors which are a fundamental aspect of the model. Therefore, we will use the established notation of the Dobzhansky-Muller model in the following.

2.2.3 Dobzhansky-Muller Model

The Dobzhansky-Muller model considers two populations which emerged from a common ancestral population (*aabb*). These populations remain separated for a certain period. Meanwhile, in each population a mutant allele arises and spreads: allele A replaces a in one population and B substitutes b in the other. Thereby, the model makes no assumptions about how new alleles have evolved, whether by selection or drift (see below). After the diverging process is completed, one population consists of individuals of genotype $AAbb$, whereas the other population is homogeneous for $aaBB$-organisms. When populations restore secondary contact via migration, the occurrence of A and B in one individual can lead to hybrid dysfunctions (Table 2.1). A and B neither have deleterious effects on their own species background nor are deleterious by themselves. But as they have not been tested together in one individual before, they might cause hybrid dysfunctions in the presence of each other (Dobzhansky, 1937, Orr, 1995; 1996). In terms of fitness landscapes, viable or optimal genotypes are connected by a viable intermediate genotype. $AAbb$ can evolve from $aabb$ through $Aabb$ without passing any deep adaptive valley. Individuals $aabb$, $Aabb$ and $AAbb$ are neither reproductively isolated nor do they suffer from stertility or inviability. This applies likewise to genotypes $aabb$, $aaBb$ and $aaBB$. Note that in contrast to the one-locus two-alleles formalization, individuals that would produce unfit hybrids are connected via several fit genotypes and are not separated by an adaptive valley.

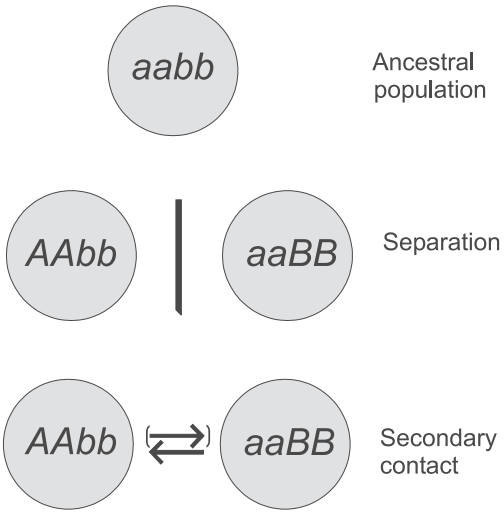

Figure 2.6: The Dobzhansky-Muller model. An ancestral population *aabb* becomes separated into two geographically isolated parts. During separation, in each population a mutant allele A or B occurs and spreads. When secondary contact starts and populations are reconnected by migration, hybrids from interspecific matings can suffer from incompatibilities between A and B.

The Dobzhansky-Muller model also explains why nuclear incompatibilities are asymmetric, at least initially (Orr, 1995). In this context asymmetric means that A and B are incompatible, whereas a and b are compatible. Since a and b represent the ancestral genotype, they should not cause hybrid dysfunctions during secondary contact. Nuclear incompatibilities should only occur between alleles that have not occurred together before. Theoretically,

one could assume that in the Ax-population additionally b is replaced by a mutant allele $\bar{B}$. Since $\bar{B}$ has not co-occurred with a, these alleles can also cause incompatibilities. In such a scenario with subpopulations $AA\bar{B}\bar{B}$ and $aaBB$, hybrid dysfunctions would be caused by incompatibilities between A and B as well as between a and $\bar{B}$. To generalize, also by considering more than two loci, nuclear incompatibilities typically occur between ancestral and derived alleles or between two derived alleles, but should not occur between two ancestral alleles.

Two- versus Multi-Loci Incompatibilities

Lots of introgression and crossing experiments (predominantly from *Drosophila* and Lepidoptera species) underline the importance of the Dobzhansky-Muller model and many cases of complementary gene interactions causing hybrid lethality or sterility are documented (Presgraves, 2002, Coyne and Orr, 1989). Thereby, the number of genes involved in incompatibilities is not restricted to only two as in the theoretical Dobzhansky-Muller model. On the contrary, it has been assumed that one pair of incompatible genes has only weak effects; sterility or inviability should rather occur by the accumulation of many incompatible loci, but how many loci are required to cause hybrid inviability or sterility remains an open question. Genetic analyses of postzygotic isolation between closely related species yield various results, and the number of genes involved can range from 2 to 190 (for review see Coyne and Orr (2004), pp. 302).

Recently, evidence for few-loci incompatibilities rose with the detection of so called speciation genes (Ting et al., 1998, Presgraves et al., 2003, Brideau et al., 2006). Brideau et al. (2006) identified the first 'Dobzhansky-Muller' genes, meaning the identification of one pair of genes causing hybrid lethality in matings between the two sister species *Drosophila simulans* and *D. melanogaster*.

	aa	aA	AA
bb	1	1	1
bB	1	$1 - hl_{\mathrm{NI}}$	$1 - h_{AB}l_{\mathrm{NI}}$
BB	1	$1 - h_{AB}l_{\mathrm{NI}}$	$1 - l_{\mathrm{NI}}$

Table 2.1: Two-loci Dobzhansky-Muller incompatibilities. The parameter l_{NI}, the NI level, describes the incompatibility level, whereas the parameters h and h_{AB} describe the dominance level of incompatible alleles. For example, we refer to recessive incompatibilities if $h = h_{AB} = 0$ and dominant incompatibilities if $h = h_{AB} = 1$.

The determination of the number of genes causing hybrid incompatibilities bears a general problem: if several incompatible genes are involved in the expression of hybrid incompatibil-

ities, this does not necessarily mean that all genes were initially there to cause hybrid dysfunctions. It is possible that subdividions of one ancestral species become reproductively isolated to a certain degree due to two-loci incompatibilities. These might provide a precondition for further genetic divergence so that thereupon stronger isolating mechanisms involving multiple loci are developed. Retrospectively, one cannot always determine how many genes started the speciation process. Beyond doubt, if reproductive isolation between groups of a common ancestral species is (nearly) complete, many incompatible gene pairs contribute to increased mating barriers. Nevertheless, it is possible that one single pair of complementary genes is sufficient to start the splitting process.

Selection or Drift?

In the Dobzhansky-Muller model two alleles that have evolved in allopatry in subdivisions of an ancestral population can lead to hybrid incompatibilities and thus initiate a speciation process. The model makes, however, no restriction on whether the new alleles have evolved by selection or drift. This question has been under debate a long time, and still there are supporters for both theories, neutralism and selectionism (Nei, 2005, Coyne and Orr, 2004).
In principle, alleles that potentially cause incompatibilities can evolve by both, drift and positive selection. Theoretical models show that neutral evolution is much slower and further more likely to happen in small populations (Nei and Wu, 1983): Assume that a mutant allele that has neither positive nor deleterious effects arises in a population. In a strongly deterministic system, it would neither in- nor decrease in frequency. In stochastic models, however, there is a certain probability that a neutral mutant in- or decreases in frequency in subsequent generations. As a result, there is a small, but positive probability that neutral or even deleterious alleles increase in frequency repeatedly from one generation to the next and finally spread to fixation in a population. In contrast, alleles that are positively selected should generally spread to fixation (although it is possible that an advantageous allele goes to extinction in a stochastic model): If a group of individuals populates a new environment, it seems plausible that mutations that bestow their carrier a fitness advantage in that niche are more likely to increase in frequency and finally become fixed in the population. In this case, reproductive isolation is a byproduct of local adaption. This makes especially sense when, as in the Dobzhansky-Muller model, populations diverge in allopatry. Laboratory experiments by Dodd (1989) showed that groups of *Drosophila pseudoobscura*

easily evolve reproductive barriers when reared on different patches with different nutrition. In contrast, matings between groups from patches with the same nutrition did not show hybrid incompatibilities, although the separation period was equal for all groups. These results suggest that the environmental differences strongly promote the evolution of reproductive isolation. Furthermore, studies on single speciation genes suggest that these genes evolved by positive selection (Presgraves et al., 2003, Ting et al., 1998). To conclude, there is lots of support for the idea that new genes arise and, if beneficial in the particular environment, natural selection promotes their spread until fixation.

2.2.4 Haldane's Rule

Hybrid incompatibilities as sterility and inviability do not need to affect every individual within one brood to the same degree. In particular, it can happen that only one sex is expressing dysfunctions. So did Haldane (1922) observe that:

> *"When in the offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous [heterogametic] sex."*

This striking phenomenon was not predicted by theorists before, it was simply an observation. These asymmetric hybridizations have been observed in various species, suggesting that speciation by postzygotic isolation could happen similarly in different animal groups. That Haldane's rule is a general pattern is further supported by its detection in taxa in which males are the heterogametic sex as in *Drosophila* and mammals, as well as in Lepidoptera and birds with heterogametic females (Coyne, 1992, Laurie, 1997). Lepidoptera and birds have a ZW sex determination system, females (ZW) are heterogametic whereas males (ZZ) are homogametic. In *Drosophila* and mammals, females are homogametic with two X chromosomes, while males carry one X and one Y chromosome. The causes why in cases of asymmetric hybridizations the heterogametic sex suffers disproportionately more often than the homogametic sex are not fully understood yet, at least a general theory explaining Haldane's rule, for both inviability and sterility in both kinds of taxa, with heterogametic males or females, is still lacking. However, there are several theories that provide plausible explanations for parts of the pattern as well as experiments that support or vitiate certain theories. Probably, Haldane's rule cannot be explained by one single theory. It is more likely that different explanations apply to different scenarios: hybrid sterility

Group	Phenotype	Asymmetric Hybridizations	Number obeying Haldane's rule
Heterogamtic males			
Drosophila	Sterility	114	112
	Inviability	17	13
Mammals	Sterility	25	25
	Inviability	1	1
Heterogametic females			
Lepidoptera	Sterility	11	11
	Inviability	34	29
Birds	Sterility	23	21
	Inviability	30	30

Table 2.2: The strength of Haldane's rule (reproduced from Coyne (1992))

and inviability might be caused by different factors as well as hybrid incompatibilities in ZW or XY taxa might occur for varying reasons. Below, we present four theories attempting to explain Haldane's rule: the dominance theory, the faster-male theory, the faster-X theory and the meiotic drive theory. The dominance theory is elaborated in detail. There is much evidence for the dominance theory in general and to explain hybrid lethality in particular and will therefore provide the basis for the theoretical model presented in chapter 4.

The Dominance Theory

Muller's dominance theory is a simple extension of the Dobzhansky-Muller model. Whereas in the original model hybrid dysfunctions occur because of incompatible autosomal loci, Muller (1942) considered an incompatibility between an X-linked and an autosomal gene to explain Haldane's rule: Assume an ancestral population with genotype $aaxx/aax$[1]. Here, a denotes an autosomal allele as in the typical Dobzhansky-Muller model while x denotes an allele located at the X chromosome (or the Z chromosome in taxa with ZW sex determination). This population becomes separated into two isolated parts. In concordance wih the original Dobzhansky-Muller model, in each population a new allele A or X evolves and replaces a or x, respectively. When populations restore secondary contact, offspring arising from interspe-

[1]homogametics/heterogametics; the other sex chromosome is ignored

cific matings can suffer from incompatibilities between A and X (like in the original model, A and X have not been tested in one individual before). If individuals AAx of one population mate with organisms $aaXX$ of the other, offspring aAX and $aAxX$ are produced. The latter are affected by incompatibilities if A as well as X act fairly dominant. But, if A is dominant and X recessive, $aAxX$-progeny remains perfectly fit whereas aAX-offspring expresses hybrid incompatibilities. Muller emphasized (based on observations in *Drosophila* crossing experiments), that hybrid males are affected by all X-linked *speciation genes*, female hybrids only by the subset of genes that are partly or fully dominant. As a result, hybrid males should suffer from hybrid dysfunctions to a greater extent than female hybrids. Obviously the same applies to ZW taxa, where females suffer more often from incompatibilities than males. This describes one great advantage of Muller's theory. Theoretically, it can explain Haldane's rule in taxa with XY and ZW sex chromosomes for both, sterility and inviability. Based on this theory, it was predicted that also female hybrids being homozygous for the deleterious X chromosome ($AAXX$ or $aAXX$) should suffer from the same hybrid dysfunctions as hybrid males (aAX). Coyne (1985) tested this prediction in three *Drosophila* species that produced sterile sons in interspecific matings. He found that females homozygous for the deleterious X chromosome remained fertile. This result was confirmed by further tests in different *Drosophila* species, where hybrid females remained fully fertile in contrast to nearly complete sterile males. Coyne's experiments further suggested that hybrid males might be sterile due to incompatibilities between X- and Y-linked loci and not because of an X-autosomal imbalance. Muller's theory thus seemed to be falsified. On the other hand, all tests refered to cases of hybrid sterility in *Drosophila*. The dominance theory could be excluded as a general theory explaining theory of Haldane's rule, but it could not be ruled out that Muller's theory still holds as an explanation of Haldane's rule in other taxa or for hybrid inviability. Indeed, Orr (1993) performed crossing-tests with *Drosophila* species, in which inviable hybrid males were produced. Orr found that females with a homozygous X on a hybrid background were inviable as well. It was even observed that females died at the same developmental stage as males, implying that the same genes cause male as well as female inviability. Further mutation experiments reinforced why hybrid sterility and inviability should be investigated separately (Orr, 1993, Wu and Davis, 1993): Genes causing hybrid sterility in males do not have effects on fertility when occurring in female hybrids and vice versa. Genes causing inviability, however, act equivalently in both sexes. Muller's theory could thus be resurrected and remains a plausible explanation of Haldane's rule, at least for inviability in taxa with heterogametic males and there is also strong evidence that Muller's theory

applies to cases of female hybrid incompatibilities in Lepidoptera species (Jiggins et al., 2001b, Salazar et al., 2004). Mathematical models of Muller's dominance theory (Orr, 1993, Turelli and Orr, 1995) revealed that speciation genes need to act, on average, recessive for heterogametics suffering more frequently than homogametics. Together with introgression (True et al., 1996) as well as backcross experiments (Coyne, 1992, Orr, 1992) implying that in fact most genes involved in incompatibilities act recessively, there is still strong support to consider Muller's dominance theory as a major explanation of Haldane's rule.

Faster-Male Theory

The faster-male theory is based on the idea that genes afflicting heterogametic hybrids evolve faster than genes afflicting homogametic hybrids. This was suggested by Wu and Davis (1993), referring to spermatogenesis being a very sensitive process and sexual selection causing faster evolution of genes expressed only in males. In fact, insects male genitalia are the fastest evolving morphological characters (Eberhard, 1985). Introgression experiments (Hollocher and Wu, 1996, True et al., 1996) showed that the faster-male theory can be considered a plausible explanation for Haldane's rule, at least for hybrid sterility in *Drosophila*. Further support for the faster-male theory comes from Michalak and Noor (2003): Using *Drosophila* microarray, they showed that genes, normally expressed in males only, are the most likely to be misexpressed on a hybrid background.
Although there is strong experimental support for the faster-male theory, it lacks two important properties: the faster-male theory cannot explain why hybrid inviability affects heterogametics only, because, in contrast to genes causing sterility, there is no evidence that there is any difference between the genes causing male or female inviability. Furthermore, the faster-male theory fails in explaining Haldane's rule in birds and Lepidoptera with heterogametic females.

Faster-X Theory

The faster-X theory was suggested by Charlesworth et al. (1987), based on the assumption that X-linked genes evolve faster than autosomal genes. This is because many mutations act recessively and thus accumulate faster on the X chromosome. Empirical data, however, show oppositional results: While Musters et al. (2006) support the faster-X theory, introgression experiments

by Hollocher and Wu (1996), Betancourt et al. (2002) and Thornton et al. (2006) do not providence evidence for X chromosomes evolving at faster rates than Y chromosomes. However, all experiments have been conducted with *Drosophila* species. It is not clear if the W chromosome in birds or butterflies behaves equally. Furthermore, the faster-X theory alone cannot explain Haldane's rule but requires dominance to yield asymmetric hybridization to the disadvantage of the heterogametic sex.

Meiotic Drive

The idea that selfish genetic elements play a role in evolution has received a lot of attention (Cosmides and Tooby, 1981, Hurst and Pomiankowski, 1991). Infectious endosymbionts, transposable elements or meiotic drive factors can be interpreted as selfish genetic elements. The latter might cause reproductive isolation in general and Haldane's rule specifically (Frank, 1991, Hurst and Pomiankowski, 1991). The underlying idea is that meiotic drive alleles distort the Fisherian sex-ratio to their own advantage. Since there is selection for a 1:1 sex-ratio in natural populations, suppressor genes would be favored and thus coevolve with meiotic drive elements. In two allopatric populations different meiotic drive factors as well as suppressor genes might evolve and in both populations the Fisherian sex-ratio would be maintained. But if these populations were to hybridize, a meiotic drive element of one population and a suppressor of the other occurring in one individual can cause hybrid incompatibilities as well as sex- ratio distortions. Introgression experiments support this theory (Tao et al., 2001). However, it is not proven that there was really this coevolution of meiotic drive and the corresponding suppressing elements (Coyne and Orr, 2004, Dermitzakis et al., 2000). Furthermore, such a scenario could just be a special case of an X-autosomal imbalance.

All presented theories contribute to explain the phenomenon of Haldane's rule, but none fulfills the qualifications to be the only explanation applicable to all cases. There is stronger support for two of them, the dominance and faster-male theory (Orr, 1997, Presgraves and Orr, 1998). While most experiments were performed on *Drosophila* species, Presgraves and Orr (1998) designed a comparative study in mosquitos to contrast faster-male and dominance theory. The two mosquito groups *Aedes* and *Anopheles* are predestined for comparisons of faster-male versus dominance theory. *Aedes*

mosquitos have a single locus sex determination: although females have XX and males XY sex chromosomes, X and Y are homolog[2] and differ only at one locus for sex determination. In contrast, *Anopheles* have degenerated Y chromosomes and X-linked genes that show normal hemizygous expression. The dominance theory should not apply to *Aedes*, because X and Y chromosome are homolog, whereas the faster-male theory could apply to both groups causing hybrid sterility. One would therefore expect hybrid male sterility to occur in *Aedes* and both, hybrid male sterility and lethality in *Anopheles*. Moreover, a bigger proportion of *Anopheles* hybrid males should suffer from dysfunctions because both factors, dominance and faster-male evolution might contribute. These predictions were indeed confirmed by crossing experiments within both groups.
Finally, we state that there is strong support for the dominance theory and the faster-male theory. Faster-X evolution and meotic drive might be the cause for certain cases of Haldane's rule, but a general validity has not been proven. Faster-male evolution doubtlessly contributes to hybrid male sterility in taxa with heterogametic males, and Muller's dominance theory still provides a general explanation for sterility and lethality in taxa with hetero- and homogametic males.

2.2.5 Theoretical Models of Speciation

Numerous theoretical models dealing with different issues in speciation theory have been presented in many studies. In a review article about models on speciation, Gavrilets (2003) denounced that there is no general framework for these modeling approaches. Different models can strongly differ from each other because methods and focus of interest vary. Comparing results from different studies is thus complicated, making it also very difficult to specify or summarize previous results from theoretical models in speciation theory.
In another review article, Hayashi and Kawata (2002) reviewed theoretical models of postzygotic isolation. However, models are categorized only regarding how reproductive isolation (RI) genes causing hybrid incompatibilities have evolved: beneficially, neutrally or deleteriously. Numerous other aspects were not addressed. For example, the form of gene interactions between populations is modeled in form of (with few exceptions) either epistatic interaction between (mostly) two loci or truncation selection involving generally more loci. Thereby, truncation selection defines fitness stepwise de-

[2]here, homolog means that X and Y carry homologous sets of genes apart from the locus for the sex determination

pending on genotype or genetic distance to be either one or zero (fully fit or dead). Epistatic selection generally provides the possibility to analyze dynamics depending on a so called incompatibility level, a parameter determining to which degree incompatible alleles affect hybrids (Wagner et al., 1994), although it is also possible to define fitness to be one or zero in such a model. Two further fundamental modeling approaches are to be distinguished: finite population sizes incorporating stochastic effects (Nei and Wu, 1983) and the modeling of individual frequencies in infinite populations in a deterministic model (Gavrilets and Gravner, 1997). These different model variants can lead to different outcomes regarding the spread of mutant alleles. Neutral or deleterious mutations can spread in the first, but do not in the second model type. Analogously, beneficial mutations spread in deterministic models, but can go to extinction in finite population models. Also the spatial structure can differ. Some models concentrate on conditions under which mutant alleles evolve in single populations. Others incorporate spatial effects, i.e. contact zones of two or more populations that have diverged in allopatry. Usually, two populations that exchange individuals via migration as in the standard Dobzhansky-Muller model are considered (Gavrilets et al., 1998). Moreover, the number of RI genes can vary. There are models that focus on determining the number of genes that is needed to cause phenotypic effects as inviability or sterility (Orr, 1995). These models obviously have to incorporate potential interactions between many loci, and the number of loci might be variable. When the number of loci causing nuclear incompatibility is not of direct interest but a necessary feature in the model, mostly the typical Dobzhansky-Muller two-loci two-allele incompatibilities are used (Nei and Wu, 1983, Wagner et al., 1994).
In models with at least two populations, it is possible to includ local effects. For example, different alleles can be differently selected in different populations or environments. In some models, however, local selection has to be included in order to maintain genetic divergence causing hybrid incompatibilities. In this case, selection is rather a technical feature without which analysis was not possible. There are, of course, studies in which local effects are of direct interest. For example, (Gavrilets, 2000) incorporated local selection in a mainland-island model. A mainland-island model describes two populations. A large mainland population and a smaller island population that receives immigrants from the mainland. In his model, immigrating alleles are positively selected on the island. He concludes that waiting time to speciation (i. e. extinction of residents) is then reduced. This is an intuitive result because in contrast to the model without local selection, immigrating alleles benefit from better local adaptation and spread faster in the island. Bordenstein and Drapeau (2001a) firstly incorporated local effects (genotype-

environment interactions (GEI)) in the classical Dobzhansky-Muller model. It is assumed that alleles being incompatible in a certain environment, can be compatible in another. In a two-population model this implies that hybrid incompatibilities are only expressed in one population but not in the other. Thereby, the assumption of the standard Dobzhansky-Muller model that NI are asymmetric can be neglected because one can suppose that ancestral alleles a and b are compatible in their original environment, but cause incompatibilities in another environment.
Studies above mainly focused on the evolution of RI genes: conditions, under which RI genes evolve, how fast they evolve or what influence spatial structures can have. But there are further models (including those in this work) in speciation theory that take the existence of RI genes for granted and investigate their stability in face of migration in parapatric populations (Spirito and Sampogna, 1995), their effect on gene flow between populations (Gavrilets, 1997) or the evolution of female mating preferences, i.e. reinforcement (Servedio and Kirkpatrick, 1997).

Stability of Isolating Mechanism: The Critical Migration Rate

Spirito and Sampogna (1995) studied under which conditions pre- and postzygotic isolating mechanisms can be established in parapatric populations. When one population separates as in the Dobzhansky-Muller model, subgroups diverge genetically and are connected via migration again, it can happen that genetic divergence is maintained but it is also possible that one new genotype vanishes again. This is an important difference because in order to establish reproductive isolation between subgroups of an ancestral species, genetic divergence must persist because with the collapse of genetic divergence the isolating mechanism is lost. The critical migration rate provides a measure for the stability of isolating mechanisms. For any set of parameters, the maximum amount of migrants for which genetic divergence is maintained is determined. If the migration rate exceeds this critical migration rate, genetic divergence and thus the isolating mechanism is lost. Thereby, the isolating mechanism is interpreted to be more stable the higher the critical migration is, i.e. the more gene exchange can happen between diverged populations. Spirito and Sampogna (1995) modeled postzygotic isolation in two different ways: selection acts against heterozygous individuals (for one- and two-loci models) or directly favors one allele at one loci. The structure of their two-loci two-allele incompatibilities correspond to the Dobzhansky-Muller incompatibilities. However, in the model by Spirito and Sampogna (1995) also alleles a and b would be incompatible (in addition to A and B), thus reproductive isolation is much stronger (see Appendix B).

This contradicts most empirical data. Expermients yielded that nuclear incompatibilities usually act asymmetrically (only A and B are incompatible). Results obtained from this theoretical study show critical migration rates of at maximum 88%. One reason is the symmetry in two-loci incompatibilities which results in higher critical migration rates than asymmetric incompatibilities. When modeling one-locus incompatibilities, (Spirito and Sampogna, 1995) assign one allele a selective advantage over the other. The chosen parameter values of the selection coefficients are, however, much higher than in other comparable studies and therefore such high stability is obtained. Due to the choice of the values of the selection coefficients and the way of modeling nuclear incompatibilities, critical migration rates are generally high, which implies a high stability of genetic divergence.
In chapter 3 we will analyze the typical Dobzhansky-Muller incompatibilities and show that under supposably more realistic conditions, i.e. asymmetry of NI and less benefit from selection, genetic divergence is less stable than suggested by Spirito and Sampogna (1995).

Gene Flow Reduction by Dobzhansky-type Incompatibilities

Gavrilets (1997) implemented the typical two-allele two-loci incompatibilities as suggested in the original Dobzhansky-Muller model, where only A and B are incompatible, while a and b do not cause hybrid incompatibilities (Table 2.1). In this model multiple populations connected via migration are considered (stepping-stone model) and gene flow reduction due to epistatic selection against hybrids is analyzed. Since two alleles at each loci are considered, the model fulfills a diploid genetic architecture that is consistent with most organisms. Gavrilets (1997) stated that in a two-population model, there is no stable equilibrium for the coexistence of all four alleles, i.e. a reproductive isolating mechanism cannot be maintained. In conclusion, it seems that completely asymmetric nuclear incompatibilities (a and b perfectly compatible) are lost when secondary is established, but symmetric NI can be maintained up to a certain critical migration rate (Spirito and Sampogna, 1995) (and see Appendix B).
To quantify the strength of gene flow reduction, so called effective migration rates can be determined. NI reduces the proportion of foreign genes coming via migrants in a population. The effective migration rate is defined as the migration rate in a model with NI that leads to the same amount of foreign genes in a model without incompatibilities. Since NI reduce gene flow, the effective migration rate must range between zero and the real migration rate m, so that $0 \leq m_{eff} \leq m$. Gavrilets (1997) showed that numerical

results match the analytical solution by (Bengtsson, 1985) and the following approximation for the effective migration rate holds:

$$m_{eff} = m\frac{(1 - h_{AB}l_{\mathrm{NI}})(3 + hl_{\mathrm{NI}})}{(3 + h_{AB}l_{\mathrm{NI}})(1 + hl_{\mathrm{NI}})}. \tag{2.1}$$

Remarkably, this formula implies that completely recessive incompatibilities ($h = h_{AB} = 0$) have no effect on gene flow since equation 2.1 becomes $m_{eff} = m$. In contrast, for dominant NI ($h = h_{AB} = 1$) we obtain $m_{eff} = m\frac{1-l_{\mathrm{NI}}}{1+l_{\mathrm{NI}}}$. Depending on the parameter l_{NI}, dominant NI can thus reduce and, if $l_{\mathrm{NI}} = 1$, complete impede gene flow.

Reinforcement

Reinforcement denotes the evolution of female mating preferences in secondary contact between diverged populations. Female mating preferences are expressed when females prefer a certain type of males as mating partners over others in order to avoid hybrid dysfunctions of offspring. Servedio and Kirkpatrick (1997) investigated how postzygotic isolation by NI interferes with the spread of these mating preferences, i.e. the evolution of prezygotic isolation. Intuitively it is clear that a trait causing females to avoid matings with partners they produce less offspring with, should spread in a population since females with this trait have a higher number of progeny than females not showing this trait. Servedio and Kirkpatrick (1997) used one-allele two-loci incompatibilities representing a simplified haploid model version. They consider two subpopulations described by M_1N_1 and M_2N_2. The recombined genotypes M_1N_2 as well as M_2N_1 emerging from interpopulation matings are supposed to be less fit. This way of modeling nuclear incompatibilities does not incorporate possible dominance effects of incompatible alleles. It further assumes symmetry of incompatibilities (M_1 incompatible with N_2 and M_2 incompatible with N_1) and does thus not represent very realistic dynamics. However, the main interest of this study is to investigate the evolution of female mating preferences and a simplified modeling approach is justified in order to reduce computational complexity.
For the spread of such mating preferences it is required that nuclear incompatibilities are maintained when secondary contact starts. In particular, such preferences evolve at a faster rate the more gene exchange between populations takes place. This follows intuitively because being choosy is more beneficial when the probability for matings with the wrong males is high. Therefore the stability, i.e. the critical migration rates of nuclear incompatibilities have direct effects on the evolution of premating isolation. First of

all, critical migrations rates must be positive numbers in order to allow preferences to spread. Moreover, high critical migration rates provide conditions under which these preferences can spread rapidly. It will thus be necessary to determine critical migration rates for realistically implemented nuclear incompatibilities in order to determine when reinforement is possible and how fast mating preferences can spread in the certain scenarios.

All three aspects, stability of isolating mechanism, gene flow reduction and reinforcement, have also been analyzed in order to investigate the impact of *Wolbachia*-induced CI on speciation processes of their hosts. The regarding models will be introduced and discussed in the next section *Wolbachia* and speciation.

2.3 *Wolbachia* and Speciation

2.3.1 Can *Wolbachia* influence Speciation Processes of their Host?

Wolbachia can manipulate the reproductive system of their hosts in various ways (section 2.1.4). Among these alterations, cytoplasmic incompatibility (CI) has attracted a lot of attention. Since CI acts as a postzygotic isolation mechanism between differently infected populations, it was suggested that *Wolbachia* influence evolutionary processes of their hosts. To build a bridge to classical speciation theory, we can consider the Dobzhansky-Muller model with respect to cytotypes instead of genotypes: one population becomes separated into two isolated parts. During separation, one (or both) subpopulations become infected with (different) CI-inducing *Wolbachia*(strains). When contact between populations is restored, unidirectional (or bidirectional) CI is expressed in matings between individuals of the subpopulations and a reproductive barrier due to cytoplasmic differences is established (Fig. 2.7).
The general role of *Wolbachia* in arthropod speciation processes has been controversial (Hurst and Schilthuizen, 1998, Werren, 1998). In this chapter *Wolbachia*'s role in speciation will be discussed. Before reviewing theoretical studies on *Wolbachia*'s role in speciation, explicit examples of species or sister species are given where *Wolbachia* probably influenced speciation processes. To stress a certain generality *Wolbachia* might have in arthropod speciation, chosen examples represent major insect groups: *Nasonia* (Hymenoptera), *Drosophila* (Diptera), *Eurema hecabe* (Lepidoptera), *Diabrotica*-beetles (Coleoptera) and *Gryllus*-crickets (Orthoptera). A further example is given for a non-insect arthropod, a spider mite species *Panonychus mori*.

Nasonia

The *Nasonia*-complex consists of three sister species *Nasonia giraulti*, *N. longicornis* and *N. vitripennis*. A common ancestor of *N. giraulti* and *N. longicornis* separated from *N. vitripennis* about 800.000 years ago and divided into the two sister species about 550.000 years later. *Nasonia* are parasitic wasps that parasite pupae of certain fly species. *N. vitripennis* occurs worldwide, whereas *N. giraulti* is found in eastern, *N. longicornis* in western North America. *N. vitripennis* lives in sympatry with each of its sister species, and have even been observed to emerge from the same host pupae. It is thus likely that sister species hybridize in nature. As typical

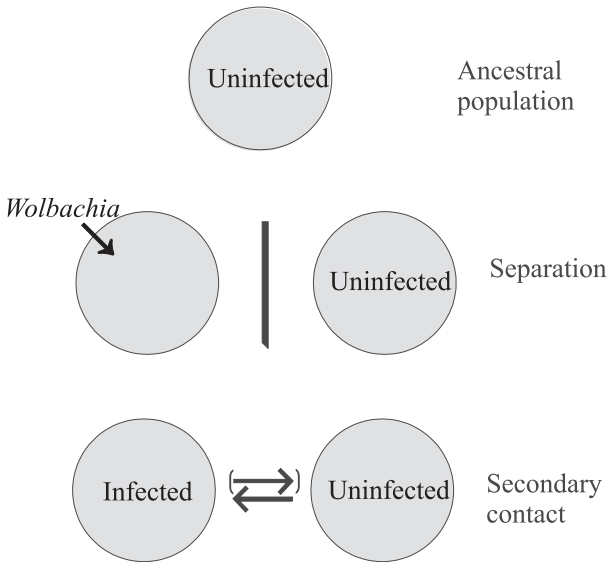

Figure 2.7: Model structure. One ancestral population is separated into two isolated parts. During separation, one population becomes infected by *Wolbachia*. When populations restore secondary contact, CI acts as a postzygotic isolating mechanism.

for Hymenoptera, *Nasonia* have a haplodiploid sex determination system. While males are haploid and develop from unfertilized eggs, diploid females originate from fertilized eggs only. Both interspecific matings between *N. giraulti* and *N. longicornis* (Bordenstein et al., 2001b) and *N. giraulti* and *N. vitripennis* (Breeuwer and Werren, 1990) have been investigated. All three sister species harbor CI-inducing *Wolbachia*. In interspecific matings, CI results in all male progeny or leads to the death of fertilized eggs. By antibiotic curing and eliminating the *Wolbachia* infection it could be shown that *Wolbachia* are the responsible factor for the incompatibilities. Breeuwer and Werren (1990) have shown that matings between *N. giraulti* and *N. vitripennis* usually produce all-male offspring. When the *Wolbachia* infection was eliminated by antibiotic treatment, the production of viable female hybrids was observed. Although hybrid females resulting from matings with cured individuals were viable, an increased mortality within their offspring was stated. This is probably due to genetic incompatibilities. Since these only occur in the F_2 hybrid generation, there is evidence that genetic deleterious effects act recessively. Bordenstein et al. (2001b) investigated matings between *N. giraulti* and *N. longicornis* and their results suggest that *Wolbachia*-induced CI occured earlier in evolution than other, genetic postzygotic isolating mechanisms. This is interesting because apparently there are several, nuclear and cytoplasmic, isolating mechanisms acting simultaneously between *Nasonia* sister species. It is possible that *Wolbachia*-induced CI allowed the genetical divergence of sister species. Even if sister species are cured from infection, incompatibilities resulting from genetic differences can maintain reproductive isolation.

Drosphila

In *Drosophila*, (unidirectional) CI was first described by Hoffmann et al. (1986) in *D. simulans*. Meanwhile, different populations of *Drosophila simulans* being uninfected, singly or doubly infected have been observed and patterns of uni- as well as bidirectional CI were found (Clancy and Hoffmann, 1996). For example, *D. simulans* populations from California and Hawaii harbor different *Wolbachia* strains that cause reproductive isolation between populations (O'Neill and Karr, 1990). Recently, Jaenike et al. (2006) studied populations of *D. recens* and *D. subquinaria* in northern USA and Canada. *D. recens* predominantly populate eastern Canada and northeastern USA, whereas *D. subquinaria* are located in western North America. There are both allopatric and sympatric areas, i.e. isolated populations of both species exist as well as an area of geographical overlap where sister species hybridize. *D. recens* is infected by a CI-*Wolbachia* with high prevalence of about 98%. In contrast, *D. subquinaria* is not infected. Matings producing less offspring due to CI are those between *D. recens* males and *D. subquinaria* females. However, the reciprocal cross leads to hybrid dysfunctions as well. *D. subquinaria* males and *D. recens* females produce viable offspring, but males are sterile. This incompatibility is supposably caused by genetic incompatibilities and shows the pattern of Haldane's rule. Interestingly, uninfected (*D. subquinaria*) females living in the sympatric area showed mating preferences in favor of males of their own species. Thereby they can prevent dysfunctions of offspring caused by genetic, but mainly cytoplasmic incompatibilities. Sympatric *D. subquinaria* females were significantly more choosy than *D. recens* females and *D. subquinaria* females in the isolated population. This indicates that *Wolbachia*-induced CI selected for the evolution of mating preferences in *D. subquinaria* females. Remarkably, *D. recens* females did not develop mating preferences although they produced sterile hybrid sons with *D. subquinaria* males. This implies that CI promotes the evolution of female mating preferences more forcefully than nuclear-based hybrid sterility.

Eurema Hecabe

Eurema hecabe, a well studied butterfly common throughout Japan, consists of two sibling species. They have not been given names yet but are called yellow (Y) and brown (B) type due to their different wing colour. Type Y butterflies populate temperate regions whereas type B populations are found in areas of tropical climate. On the subtropical Okinawa Island, there is an overlap of both populations (Kato, 2000, Narita et al., 2006). Kobayashi et al. (2001) found that asymmetrical sexual isolation in form of female mate

discrimination acts between individuals of the two sibling species from the sympatric region. However, experiments were carried out under cage conditions and it is not clear if females show the same mating preferences under natural conditions. Recently, *E. hecabe* has been found to be infected by a CI-*Wolbachia* (Narita et al., 2006). All B-type butterflies were infected, whereas in Y-type populations infected and uninfected individuals coexisted. The potential role of *Wolbachia* in speciation processes of *E. hecabe* has not been elaborated yet. Since both sibling species are infected, bidirectional CI could have played an important role as an isolating mechanism. However, as females generally refuse to mate with other males of the opposite type it is difficult to investigate postzygotic isolating mechanism as CI, nuclear-based hybrid inviability or sterility in crossing experiments.

Diabrotica Beetles

The *Diabrotica* beetles *D. v. virgifera* and *D. v. zeae* populate large areas of Mexico and the USA. Sister species usually occur in allopatry, but there are two zones of secondary contact where *D. v. virgifera* and *D. v. zeae* hybridize. Giordano et al. (1997) collected individuals of both species and tested for the presence of *Wolbachia*. Individuals from 17 sites of the Western corn rootworm *D. v. virgifera* were sampled. 15 of these populations harbored *Wolbachia* infections, and two allopatric populations were uninfected. In contrast, all populations of the Mexican corn rootworm *D. v. zeae* were uninfected. Matings between infected *D. v. virgifera* males and uninfected *D. v. zeae* females showed almost complete reproductive isolation (only 0.4% of eggs hatched). By antibiotic curing it was proven that *Wolbachia*-induced CI caused hybrid breakdown. Remarkably, there is no evidence for other isolating barriers, such as premating, temporal or ecological isolation and so far, CI has been the only isolating barrier identified.

Gryllus Crickets

Giordano et al. (1997) further analyzed six closely related *Gryllus* cricket species. These showed complex *Wolbachia* infection patterns. Only one taxon is uninfected, whereas others are singly or doubly infected. Thereby, five different *Wolbachia* strains are involved. Uni- and bidirectional mating incompatibilities between taxa were observed and the distribution of *Gryllus* taxa in North America with many areas of geographical overlap provides various possibilities of naturally occurring hybridization. Giordano et al. (1997) showed that mating incompatibilities are caused by *Wolbachia* infections and

claimed that *Wolbachia* have been an essential factor in speciation processes of their cricket hosts. Because of this very complex infection pattern, further experiments, for instance crossings with antibiotic curing and identification of other potential isolating barriers, are necessary to determine *Wolbachia*'s role in speciation processes within the *Gryllus*-complex more precisely.

Panonychus Mori

Beside insects, further arthropod species are infected by *Wolbachia*. Spider mites for example frequently express uni- and bidirectional incompatibilities. As *Nasonia*, spider mites are haplodiploid species and cytoplasmic incompatiblities are expressed through male-biased offspring. Gotoh et al. (2005) collected individuals from 25 natural populations of the spider mite *Panonychus mori* throughout Japan and performed crossing experiments to determine to which degree *Wolbachia* is involved in these incompatibilities. Five out of 25 populations harbored *Wolbachia*. The crossing experiments included infected individuals as well as cured individuals from actually infected populations so that in total $30 \times 30 = 900$ crosses were carried out. Results were complex and showed a variety of hybrid incompatibilities due to cytoplasmic incompatibilities, but also to nuclear incompatibilities between infected and uninfected, as well as uninfected and uninfected populations. It is important to note that in certain crosses not only CI, but also nuclear-nuclear interactions affect the fitness of hybrid offspring. This provides strong evidence that *Wolbachia*-induced CI and nuclear incompatibilities interact and simultaneously promote speciation processes of host species.

2.3.2 Population Biology of CI-*Wolbachia*

Wolbachia's role in arthropod speciation will strongly depend on the stability of CI as an isolating mechanism. More precisely, bacteria can only influence speciation when infection polymorphisms are maintained, i.e. when infected and uninfected (in the case of unidirectional CI) or differently infected (in the case of bidirectional CI) individuals coexist. If infected individuals spread or become extinct in a certain population, there would be no isolating mechanism any more. Therefore population dynamics of CI-*Wolbachia* have to be studied and it has to be determined if and under which conditions infection polymorphisms can exist. Several theoretical models have helped to understand population dynamics. In this part, we will shortly review theoretical studies on population dynamics of *Wolbachia*.

Single populations

Parents			Offspring	
Mother	Father	Frequency	I	U
I	I	p^2	$(1-f)$	
I	U	$p(1-p)$	$(1-f)$	
U	I	$p(1-p)$		$1-l_{\mathrm{CI}}$
U	U	$(1-p)^2$		1

Table 2.3: Mating table for unidirectional CI. Shown are possible mating types between infected (I) and uninfected (U) individuals and the emerging offspring. p denotes the frequency of uninfected individuals, f is the fecundity reduction of infected females and l_{CI} the proportion of inviable offspring in incompatible matings.

What happens, if individuals infected with a CI-*Wolbachia* invade a population of actually uninfected individuals? It is difficult to answer this question based on empirical studies. Intuitively, one would assume that infected individuals spread in a population if they have a fitness advantage over uninfected individuals. In the case of unidirectional CI, infected females can produce (infected) offspring with both, infected and uninfected males. Uninfected females, however, only produce (uninfected) offspring with uninfected males, but produce less or no progeny when mating with infected males. The advantage of infected females is therefore frequency dependent. Since for uninfected females the probability of mating with an infected male increases with increasing proportion of infected males in the population, the relative fitness advantage of infected over uninfected females increases as well with the proportion of infected individuals. This fitness advantage, however, is usually compensated for since a *Wolbachia* infection often induces certain fitness costs to their host. Infected females often suffer from fecundity reduction and produce a smaller total number of offspring than uninfected females. Furthermore, infected females can also produce uninfected progeny due to imperfect maternal transmission. Whether a *Wolbachia* infection spreads in a population or not will depend upon the strength of CI and the attendend costs. If the fitness advantage due to CI is higher than the costs of infection, *Wolbachia* infection will likely spread. If costs are higher, *Wolbachia* infection might not be established.

Several theoretical models have helped to understand the population dynamics of CI-*Wolbachia*. Caspari and Watson (1959) presented a first model approach. Population dynamics within a single population are modeled. Generations are assumed to be discrete and non-overlapping and individuals mate randomly. The model determines frequencies of infected and uninfected individuals. Assume that p describes the frequency of infected individuals. Let the infected females' fecundity be reduced by a factor f and let l_{CI} be the CI level, i.e. the proportion of inviable offspring in the incomaptible mating

Parents			Offspring	
Mother	Father	Frequency	I	U
I	I	p^2	$(1-f)t$	$(1-f)(1-t)(1-l_{\mathrm{CI}})$
I	U	$p(1-p)$	$(1-f)t$	$(1-f)(1-t)$
U	I	$p(1-p)$		$1-l_{\mathrm{CI}}$
U	U	$(1-p)^2$		1

Table 2.4: This generalized mating table extends the mating table 2.3 by the parameter t that allows imperfect *Wolbachia* transmission so that infected mother produce a certain proportion $1-t$ uninfected progeny.

between infected males and uninfected females. With Table 2.3, frequencies of infected individuals for subsequent generations can be described:

$$p' = \frac{p(1-f)}{1-pf-p(1-p)l_{\mathrm{CI}}}. \tag{2.2}$$

Fixpoints of this equation correspond to equilibrium frequencies of the population dynamics. These fixpoints p^* are easily obtained. Solving $p^* = p' = p$ yields $p_1^* = 0$, $p_2^* = 1$, and $p_3^* = \frac{f}{l_{\mathrm{CI}}}$. The first two, p_1^* and p_2^*, are stable and correspond to scenarios where all individuals are either uninfected (p_1^*) or infected (p_2^*). The third fixpoint is unstable and describes a threshold frequency. If the frequency of infected individuals is above this value, the frequency of infected individuals converges to p_2^*. The *Wolbachia* infection will thus spread until fixation while uninfected individuals become extinct. With a frequency below p_3^*, infected individuals do not succeed to spread and the infection will be lost.

The model by Caspari and Watson (1959) was generalized by Hoffmann et al. (1990) by including a parameter $t < 1$, the transmision rate, allowing for imperfect maternal transmission. It is assumed that infected females produce a certain proportion $1-t$ of uninfected offspring (Table 2.4). Then, the generalization of formula 2.2 is

$$p' = \frac{p(1-f)t}{1-pf-p(1-p)l_{\mathrm{CI}}-p^2((1-f)(1-t)l_{\mathrm{CI}})}. \tag{2.3}$$

It is easily seen that $p_1^* = 0$ is still a fixpoint. The other two equilibrium frequencies are obtained by solving the quadratic equation

$$p^2 l(1-f(1-t)) - p(1-f+l_{\mathrm{CI}}) + 1 - ft = 0.$$

Hence, the other fixpoints are

$$p_2^* = \frac{f + l_{\mathrm{CI}} + \sqrt{(f + l_{\mathrm{CI}})^2 - 4l(1-(1-f)t)(1-(1-f)(1-t))}}{2l_{\mathrm{CI}}(1-(1-f)(1-t))} \tag{2.4}$$

and

$$p_3^* = \frac{f + l_{\mathrm{CI}} - \sqrt{(f + l)^2 - 4l_{\mathrm{CI}}(1-(1-f)t)(1-(1-f)(1-t))}}{2l_{\mathrm{CI}}(1-(1-f)(1-t))}. \tag{2.5}$$

p_2^* describes the second stable fixpoint, whereas p_3^* can be interpreted again as a threshold frequency. If the infection frequency exceeds p_3^*, *Wolbachia* spreads until p_2^* is reached, otherwise the infection gets lost. The fixpoint p_2^* describes an equilibrium state at which *Wolbachia* has spread in the population. If transmission is imperfect, it generally holds that $p_2^* < 1$ because there always remains a certain proportion of uninfected individuals. The final frequency of *Wolbachia* will depend on the values of the different parameters. Both, f and t are supposed to be only slightly smaller than 1 (Hoffmann et al., 1990). In contrast, the CI level can range from weak CI in *Drosophila* (Hoffmann et al., 1990) to complete CI in Nasonia (Breeuwer and Werren, 1990). Still, *Wolbachia* infections are supposed to reach high frequencies within populations. If we assume $f = 0.95$ and $t = 0.95$ *Wolbachia* reach fixation for complete CI $l_{\mathrm{CI}} = 1$ and still a frequency of 80% if CI is rather weak with $l_{\mathrm{CI}} = 0.3$.
These theoretical findings led to the assumption that *Wolbachia* cannot perceptibly influence speciation processes. Since the *Wolbachia* infection either spreads almost to fixation or becomes extinct, CI would not act as a postzygotic isolation mechanism. However, recently it was shown by other theoretical studies that the coexistence of differently infected as well as uninfected and infected individuals is possible in structured populations.

Structured Populations

Within recent years, population dynamics of CI-*Wolbachia* in structured populations have been examined (Telschow et al., 2005b, Flor et al., 2007). The Dobzhansky-Muller model provided the basic structure of the theoretical models. In analogy to the original model where one ancestral population splits into two subpopulations which diverge genetically, it is assumed that instead of genetical changes, one population aquires a CI-inducing *Wolbachia* infection during separation (Fig. 2.7). With the beginning of secondary contact, unidirectional CI acts as an isolating mechanism between the populations due to incompatible matings between infected males and uninfected

females. Alternatively, both populations can become infected by different CI-inducing *Wolbachia* strains (Fig. 2.8) so that bidirectional CI is expressed in interpopulation crosses. Like in studies considering only one population, the point of interest regarding speciation processes is under which conditions the isolating mechanism is maintained. For unidirectional CI this means the coexistence of infected and uninfected individuals. For bidirectional CI it has to be studied when the coexistence of indiviuals infected with different *Wolbachia* strains is possible. In the theoretical studies presented and throughout this work it will be assumed that an individual is infected by one *Wolbachia* strain only, although double infections can occur (Narita et al., 2007).

Telschow et al. (2005b) have shown that two *Wolbachia* strains can stably coexist under a wide range of conditions, substantiating *Wolbachia*'s potential role in the evolution of their hosts. Regarding unidirectional CI, Flor et al. (2007) found that the coexistence of infected and uninfected individuals is possible. However, the conditions under which infection polymorphisms occur are much more restricted than for bidirectional CI. To evaluate and compare the stability of infection polymorphisms, critical migration rates for the particular isolating mechanism can be determined.

	Father	
Mother	W_0	W_1
W_0	1	$1 - l_{\mathrm{CI},1}$
W_1	$1 - l_{\mathrm{CI},0}$	1

Table 2.5: Bidirectional CI. Mating partners carrying different Wolbachia strains produce a reduced numer of offspring. When mating partners carry the same strain, no incompatibilities occur .

Stability of CI: The Critical Migration Rate

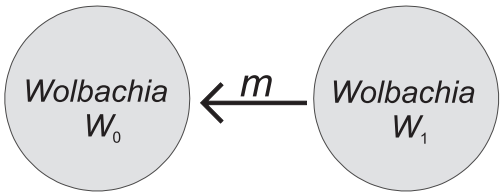

Figure 2.8: This graph describes a scenario where two isolated populations acquire different CI-*Wolbachia* infections. When populations restore secondary contact, bidirectional CI acts as a postzygotic isolating mechanism.

The term of the critical migration rate was already introduced in section 2.2.5. In the regarding context, the critical migration rate described the maximum value of the migration rate between parapatric populations below which genetic divergence and thus nuclear incompatibility as an isolating mechanism was maintained. Equivalently, critical migration rates for the coexistence of infected and uninfected individuals or differently infected individuals and thus for the maintenance of uni- or bidirectional CI as isolating mechanisms can be determined (Tel-

schow et al., 2005b, Flor et al., 2007). Telschow et al. (2005b) considered a scenario as described in Figure 2.8. Frequencies of individuals infected with either strain W_0 or strain W_1 are modelled. The model formalizes the individuals' lifecycle consisting of a migration and a reproduction step. In the reproduction step, CI is expressed as described in Table 2.5. In concordance with models for single populations, generations are supposed to be discrete and non-overlapping and mating is random. The migration step describes the migrating behavior in the model, where a certain fraction m of one population is replaced by individuals of the other population. It is distinguished between models with migration in one direction (mainland-island models) and models with migration in both directions (two-way migration models). Let p denote the frequency of individuals infected with strain W_0 in the population receiving migrants infected with strain W_1. The frequency of W_0 for subsequent generations can then be described by the following equation

$$p' = \frac{(1-m)p(1-l_{\mathrm{CI},1}(1-p))}{1-(l_{\mathrm{CI},0}+l_{\mathrm{CI},1})p(1-p)}. \tag{2.6}$$

This system was analyzed (analytically and numerically) by Telschow et al. (2005b). It was found that, for any given CI level, both *Wolbachia* strains can stably coexist if migration is below a certain threshold value which is referred to as the critical migration rate. If migration exceeds this value, strain W_1 spreads and strain W_0 becomes extinct. The critical migration rate can be displayed as a function of the CI levels

$$m_c = \frac{2(l_{\mathrm{CI},0}+l_{\mathrm{CI},1}) - l_{\mathrm{CI},0}l_{\mathrm{CI},1} - 2\sqrt{(l_{\mathrm{CI},0}+l_{\mathrm{CI},1})^2 - l_{\mathrm{CI},0}^2 l_{\mathrm{CI},1} - l_{\mathrm{CI},0}l_{\mathrm{CI},1}^2}}{l_{\mathrm{CI},1}^2}. \tag{2.7}$$

Here, $l_{\mathrm{CI},0}$ and $l_{\mathrm{CI},1}$ denote the CI levels of the two different strains W_0 and W_1, respectively. For perfect reproductive isolation ($l_{\mathrm{CI},0} = l_{\mathrm{CI},1} = 1$), both strains coexist if migration is below 17.2%. Equation 2.7 further shows that if residents are not infected ($l_{\mathrm{CI},0} = 0$), uninfected individuals become extinct

$$m_c = \frac{2l_{\mathrm{CI},1} - 2\sqrt{l_{\mathrm{CI},1}^2}}{l_{\mathrm{CI},1}^2} = \frac{2l_{\mathrm{CI},1} - 2l_{\mathrm{CI},1}}{l_{\mathrm{CI},1}^2} = 0. \tag{2.8}$$

and all remaining individuals harbor *Wolbachia* strain W_1.
For a model with symmetric migration in both directions, the threshold value for perfect isolation ($l_{\mathrm{CI},0} = l_{\mathrm{CI},1} = 1$) is with 19.2% even higher than in the one-way migration model. If either $l_{\mathrm{CI},0} = 0$ or $l_{\mathrm{CI},1} = 0$ holds i.e. one

population is initially uninfected, the infection will spread in both populations and uninfected individuals become extinct. In both, one- and two-way migration models individuals disappear if they do not induce CI. In conclusion, there is no stable coexistence of infected and uninfected individuals in parapatric populations. Since infected individuals replace the uninfected individuals, unidirectional CI as an isolating mechanism does not persist. However, possible fecundity effects of infected females or imperfect maternal transmission have not been incorporated. By doing so, Flor et al. (2007) could show that infection polymorphisms can occur if infected females are either less fecund than uninfected females or produce uninfected progeny due to imperfect maternal transmission. Nevertheless, the maximum critical migration rates below which infected and uninfected individuals can coexist are with less than 4% relatively low. Analytical solutions for the critical migration rates are obtained for models with migration in one direction only. If migrants come from the uninfected population, the critical migration is

$$m_c = \begin{cases} \frac{(1-f-l_{\mathrm{CI}})^2}{4f} & for\ l_{\mathrm{CI}} > 1-f \\ 0 & else \end{cases}$$

and

$$m_c = \begin{cases} \frac{1+f-2\sqrt{1-l_{\mathrm{CI}}}}{l_{\mathrm{CI}}} & for\ l_{\mathrm{CI}} > 1-f \\ 0 & else \end{cases},$$

if infected individuals invade the uninfected population. For models with two-way migration, critical migration rates can be rated by the analytical solutions from the one-way migration models:

$$m_c \leq min\left\{ \frac{(1-f-l_{\mathrm{CI}})^2}{4f}, \frac{1+f-2\sqrt{1-l_{\mathrm{CI}}}}{l_{\mathrm{CI}}} \right\}.$$

Thereby, critical migration rates are highest for intermediate levels of CI. Analogously, analytical solutions or estimates for the critical migration rates are obtained for models with imperfect maternal transmission instead of fecundity reduction of infected females (for details see Flor et al. (2007)).
To summarize, we state that bidirectional CI induces strong reproductive isolation under a broad range of conditions. Unidirectional CI can act as an isolating mechanism as well, but is weaker, less robust in the face of migration and thus collapses more easily.

Gene Flow Reduction by *Wolbachia*-induced CI

A stable maintainance of a reproductive isolating mechanism induced by *Wolbachia* can have consequences on genetic divergence and gene flow be-

tween populations. *Wolbachia*'s impact on gene flow can be studied as described in section 2.2.5 where postzygotic isolation was caused by genetic factors. Equivalently to Dobzhansky-Muller incompatibilities, *Wolbachia*-induced CI reduces gene flow. Telschow et al. (2002) have found analytical approximations for the effective migration rates

$$m_{eff} = m\frac{1 - l_{\mathrm{CI}}}{1 + l_{\mathrm{CI}}}. \tag{2.9}$$

At this point we recapitulate what was found for Dobzhansky-type incompatibilities. Formula 2.1 described the effective migration rate in the Dobzhansky-Muller model

$$m_{eff} = m\frac{(1 - h_{AB}l_{\mathrm{NI}})(3 + hl_{\mathrm{NI}})}{(3 + h_{AB}l_{\mathrm{NI}})(1 + hl_{\mathrm{NI}})}.$$

Gene flow reduction is highest for dominant genetic incompatibilities, i.e. $h = h_{AB} = 1$. Formula 2.1 then becomes

$$m_{eff} = m\frac{1 - l_{NI}}{1 + l_{NI}}$$

and equals the effective migration rate for bidirectional CI (formula 2.9). Therefore, gene flow reduction by bidirectional CI is at least as strong as gene flow reduction induced by Dobzhansky-type incompatibilities.
The effect of unidirectional CI on gene flow has been investigated as well (Telschow et al., 2007). In a model where infected individuals migrate to an uninfected population, the effective migration rate can be described by

$$m_{eff} = m\frac{1 - l_{\mathrm{CI}}}{2 - f}. \tag{2.10}$$

The parameter f denotes again the fecundity reduction of infected females. For complete CI ($l_{\mathrm{CI}} = 1$), formula 2.10 shows that unidirectional CI is able to totally prevent gene flow. However, if a model with uninfected migrants and infected residents is considered, there is no barrier against gene flow. If migration takes place in both directions, a gene flow reduction could be stated to happen in both directions, too. Corresponding to the results of the one-way migration models, this reduction is asymmetric and stronger in the direction where infected individuals migrate to the originally uninfected population.
To summarize, we state that bidirectional CI reduces gene flow at least as effectively as dominant nuclear incompatibilities. Unidirectional CI can also reduce gene flow. This happens under more restricted conditions compared to bidirectional CI and is usually less strong.

Reinforcement

What we nowadays understand under the term reinforcement dates back to Dobzhansky (1937), who described a scenario in which two populations diverge in allopatry; upon secondary contact, genetic differences can lead to hybrid dysfunctions. Therefore, individuals mating with partners of their own type have a fitness advantage over individuals who do not discriminate. Servedio and Kirkpatrick (1997) presented a theoretical model examining the impact of nuclear incompatibilities on the evolution of female mating preferences (see 2.2.5).
If we now consider *Wolbachia* infections instead of genetic divergence, the same theory applies. In a unidirectional CI scenario, it is beneficial for uninfected females to mate with uninfected males. Assume that mating behavior of uninfected females is described by a certain preference trait. This preference trait divides uninfected females into two classes. Females of one class show mating discrimination against males expressing a trait that is connected with the *Wolbachia* infection. Therefore, in contrast to females that do not discriminate, they have a higher probability to mate with an uninfected male and thus produce, on average, more progeny. The trait letting females be choosy would therefore spread among uninfected females. This applies likewise to a bidirectional CI scenario, with the difference that females should choose a mating partner infected with the same *Wolbachia* strain.
Theoretical studies have shown that both, bi- and unidirectional CI promote the spread of these female mating preferences (Telschow et al., 2005a; 2007). Thereby, the rate of spread partly depends on the amount of incoming migrants. This is intuitively interpreted: the higher the fraction of incompatible males, the stronger the need for females to discriminate. Thus, if the isolating mechanism is stable up to high migration rates, female mating preferences spread more rapidly. As shown above, bidirectional CI can be maintained up to higher migration rates than unidirectional CI and bidirectional CI therefore allows faster evolution of mating preferences than unidirectional CI.

2.3.3 Summary and Outlook

The impact of *Wolbachia* on speciation processes of their hosts has been controversial (Werren, 1998, Hurst and Schilthuizen, 1998). For bidirectional CI, there is strong theoretical and empirical evidence that *Wolbachia* can promote speciation processes of their hosts (Breeuwer and Werren, 1990, Telschow et al., 2002; 2005a). However, bidirectional CI has been argued to occur rarely in nature and is therefore not qualified to be considered a general factor in arthropod speciation. Unidirectional CI is generally supposed

to occur more frequently because it requires only one population to acquire an infection. However, unidirectional CI does not seem to be a strong force in all three speciation-related aspects discussed above, it is at least less effective than bidirectional CI. Comparing unidirectional CI's influence with the impact of genetic incompatibilities regarding reinforcement or critical migration rates in the three discussed scenarios is complicated. In the corresponding studies genetic features were mostly formalized in a simplified haploid model structure and are thus not representing genetic systems of most considered organisms. In the following work, we will present models that show a diploid genetic structure. By doing so, we can implement genetic features more realistically and further draw better comparisons between the impact of *Wolbachia* on the one hand and nuclear incompatibilities on the other hand. Furthermore, we can identify differences arising from haploid and diploid modeling.

To yield a broad acceptance of *Wolbachia* as a promoter of speciation, it has to be shown that *Wolbachia* generally influence arthropod speciation processes rather than in few special cases only. In particular, it will have to be shown that unidirectional CI plays an important role in speciation processes. So far, most theoretical models focused on either cytoplasmic or nuclear factors when investigating their impact on speciation. Some species complexes, however, have shown to express both, cytoplasmic and nuclear incompatibilities in crossings of different strains or sister species (see section 2.3.1). Moreover, it should be rather a general scenario that both, nuclear incompatibilities and *Wolbachia*-induced CI occur simultaneously. Nuclear incompatibilities are seen as a major force in speciation. If, as in the Dobzhansky-Muller, a population divides and one or both subpopulations become infected by *Wolbachia*, subpopulations should have diverged genetically as well. Therefore, when investigating *Wolbachia*'s impact on speciation processes, nuclear incompatibilities should be involved because they very likely coocured. Such scenarios will be studied in the following work. We will focus on the stability (i.e. critical migration rates) of single isolating mechanisms and how the presence of the additional mechanism affects stability of the other. In chapter four, interactions of Dobzhansky-Muller incompatibilities and *Wolbachia*-induced CI will be investigated for large parameter spaces. Due to the diploid structure of our models, we are able to analyze and contrast dynamics for recessive and dominant NI. Furthermore, we will elaborate on the effect of *Wolbachia* when inducing unidirectional and (symmetric and asymmetric) bidirectional CI. In chapter five we will examine more concrete scenarios with Haldane-type NI and focus on unidirectional CI. The concentration on unidirectional CI is justified and important because it has to be shown that unidirectional CI has the ability to drive speciation processes in order to

establish *Wolbachia* as a general arthropod speciation promoter.

Chapter 3

Interactions of Dobzhansky-Muller Incompatibilities and *Wolbachia*-induced CI

In this chapter interactions of *Wolbachia*-induced cytoplasmic incompatibility (CI) and nuclear incompatibility (NI) are investigated. We utilize the classical Dobzhansky-Muller model of speciation which is expanded by *Wolbachia* infections causing CI. Both isolating mechanisms acting simultaneously are analyzed with a focus on possible synergistic effects and are compared to models in which only one isolating mechanism is acting. By analyzing a model with NI only, we can show that genetic divergence is not maintained unless either local selection acts on the alleles involved in incompatibilities or reproductive isolation is perfect, i.e. all hybrids are inviable. Further, examining dominance effects of deleterious alleles yields that especially recessive incompatibilities are easily lost once secondary contact is established. Incorporating intracellular bacteria *Wolbachia* generally leads to an increase in stability of genetic divergence. Particularly, bidirectional CI stabilizes NI over a broad parameter range, but also unidirectional CI can reinforce genetic divergence under certain conditions. Thus, *Wolbachia* select for Dobzhansky-Muller incompatibilities and reinforce the impact of genetic incompatibilities on speciation processes.

3.1 Introduction

The Dobzhansky-Muller (Dobzhansky, 1940, Muller, 1942) model is a fundament for numerous theoretical modeling approaches concerning speciation and provides an explanation for how groups of a common ancestral species become reproductively isolated under natural conditions. The model assumes that a population characterized by two alleles at each of two loci ($aabb$) splits into two isolated groups. During separation, populations diverge genetically by the occurrence and fixation of mutant alleles. More precisely, a mutant allele A replaces a in one population while in the other the ancestral allele b is substituted by a mutant B. Afterwards, secondary contact between populations is established. When subpopulations hybridize, the common occurrence of A and B in one individual decreases hybrid fitness by causing lethality or sterility (for details see 2.2.3). It is generally accepted that speciation processes happen according to the Dobzhansky-Muller model, i.e. that nuclear divergence during separation creates a reproductive barrier between groups of a common species.
In the last decades it has been argued that speciation processes do not need to be driven by nuclear factors exclusively, but cytoplasmic elements can play a role as well (Laven, 1959, Werren, 1998). Representatives of such cytoplasmic elements are intracellular bacteria *Wolbachia*. *Wolbachia* induce a cytoplasmic mating incompatibility (see 2.1.4), that causes hybrid lethality when infected males mate with uninfected females (unidirectional CI) or when mating partners are infected with different strains (bidirectional CI). Thus, in analogy to the classical Dobzhansky-Muller model, reproductive isolation can be established by one or both populations acquiring a *Wolbachia* infection. There is increasing theoretical (Telschow et al., 2002; 2007) and empirical (Breeuwer and Werren, 1990, Jaenike, 2007) evidence that *Wolbachia* perceptibly influence speciation processes of their arthropod host. This is particularly important because *Wolbachia* might infect up to two thirds of arthropod species (Hilgenboecker et al., 2008) and could thus be considered a general factor in the evolution of arthropods.
So far, most studies on speciation processes have focused on either nuclear or cytoplasmic factors. Some examples of natural populations, however, show that coaction of cytoplasmic and nuclear reproductive isolation has likely happened in insect but also other arthropod species (see section 2.3.1). Theoretical studies on *Wolbachia*'s role in speciation use the basic structure of the Dobzhansky-Muller model (see section 2.3). It is assumed that one ancestral population separates into two parts. While being separated, subpopulations acquire *Wolbachia* infections that cause CI during secondary contact. What is usually neglected in such studies is that usually populations should si-

multaneoulsy diverge genetically, so that also genetic incompatibilities will contribute to reproductive isolation.
In this chapter we analyze theoretically autosomal-autosomal nuclear incompatibilities according to the Dobzhansky-Muller model which interact with cytoplasmic incompatibilities that arise when one or both populations become infected by *Wolbachia*. At first, the model is analyzed in the absence of bacteria. This is necessary to investigate *Wolbachia*'s impact on dynamics afterwards, but also provides new insights into the stability of nuclear incompatibilities. In contrast to previous models, we include two features: local viability selection and dominance effects of incompatible alleles. The latter requires a diploid genetic architecture. We can analyze local effects as adaptation and also contrast recessive versus dominant incompatibilities and their consequences on forming of hybrid zones during secondary contact. Afterwards, several variations of *Wolbachia*-induced CI interacting with NI are analyzed. Beginning with symmetric bidirectional CI, i.e. both population acquire *Wolbachia* that induce equal incompatibility levels, we continue with a more general case allowing different strains to express different strengths of CI. Finally, we explore unidirectional CI models where only one population becomes infected. We will show that nuclear incompatibilities are only maintained in parapatric populations if they evolved by positive selection. If local selection acts, dominant incompatibilities are maintained up to higher migration rates than recessive incompatibilities. *Wolbachia*-induced bidirectional CI significantly increases the stability of NI being most effective in stabilizing recessive NI which easily collapses otherwise. Analyzing interactions of unidirectional CI and NI will show that the isolating mechanism that is more stable can increase stability of the weaker mechanism by interacting with it.

3.2 The Model

Our model combines features from studies on the role of *Wolbachia*-induced CI and classical NI in speciation processes. In doing so, the theoretical model also adopts characteristics from models focusing on the role of *Wolbachia* (Telschow et al., 2005b) as well as from models focusing on the role of genetic incompatibilities (Gavrilets, 1997). The rough model structure is consistent with models on *Wolbachia* by Telschow et al. (2005b), Flor et al. (2007) or reinforcement models (Servedio and Kirkpatrick, 1997, Telschow et al., 2005a). This provides the possibility to compare results and can serve as a control criterion in particular. The genetic components, i.e. the modeling of nuclear incompatibilities, follows models by Gavrilets (1997) or Turelli

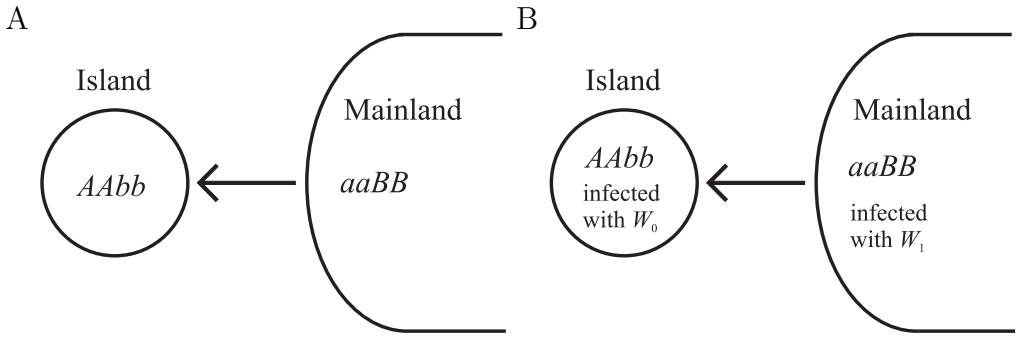

Figure 3.1: Model scenarios: Mainland-island models based on the Dobzhansky-Muller model. The island population initially consists of individuals of genotype *AAbb* and receives immigrants from the mainland characterized by genotype *aaBB*. Scenario A describes the typical Dobzhansky-Muller model, while scenarios B is extended by *Wolbachia* infections causing bidirectional CI. With the establishment of secondary contact, interpopulation matings produce less offspring due to nuclear incompatibilities (scenario A & B) and *Wolbachia*-induced CI (scenario B).

and Orr (1995) and is different from the way NI is formalized by Telschow et al. (2005a) or Servedio and Kirkpatrick (1997), who used haploid models. The basis of our model shows a diploid genetic architecture. This is important to realistically implement genetic incompatibilities and to have the possibility to investigate dominance effects of incompatible alleles.
Combining *Wolbachia*-induced CI and NI in one model allows the comparison of the different isolating mechanisms as the model can easily be analyzed incorporating only one of them, and further provides the possibility to analyze their interactions. However, due to the diploid and spatial structure our model is in some regards more complex than other models that are haploid (Telschow et al., 2005b) or without spatial structure (Turelli and Orr, 1995). Therefore, analytical solutions can only be derived for few special cases and most results are obtained from computer simulations.
Since dynamics depend on many parameters, we will restrict first investigations to mainland-island models, i.e. only the island population receives genetic influx from a mainland population. This is justified because one-way migration models are simpler to analyze and results easier to interpret. Furthermore, in the scenarios discussed below, two-way migration does not provide new insights into population dynamics. For completeness, results from two-way migration models are briefly discussed in Appendix C.

3.2.1 Model Description

The model is based on the classical Dobzhansky-Muller model of speciation (Dobzhansky, 1940, Muller, 1942): A population splits into two subpopulations that remain in allopatry for a certain period. During separation they

diverge both genetically and cytoplasmically. Genetic divergence happens by the occurrence and fixation of a mutant allele in each populations. Cytoplasmic divergence is due to one or both subpopulations acquiring a *Wolbachia* infection. Afterwards, populations experience secondary contact via migration. Offspring of interspecific matings can be inviable due to genetic incompatibility and *Wolbachia*-induced CI. The model structure follows previous models (Telschow et al., 2005a, Servedio and Kirkpatrick, 1997) and formalizes the lifecycle of individuals consisting of three steps: migration, local viability selection and reproduction. In the reproduction step individual frequencies of the next generation are determined. It is assumed that generations are discrete and non-overlapping.
Let $p_{\vec{i}}$ describe frequencies of certain individual-types in the island population. The 3-dimensional vector $\vec{i} = (i_1, i_2, i_3)$ indicates geno- and cytotype of the particular individuals. The first two entries denote the genotype. It is

$$i_1 = \begin{cases} 0 & \text{for } aa \\ 1 & \text{for } AA \\ 2 & \text{for } Aa \end{cases},$$

and i_2 is defined analogously to describe alleles at the B-locus. The third entry i_3 defines the infection status. In bidirectional CI models two *Wolbachia* strains W_0 and W_1 are involved and every individual is infected with one strain and double infections are excluded. It is

$$i_3 = \begin{cases} 0 & \text{infected with } W_0 \\ 1 & \text{infected with } W_1 \end{cases}.$$

In unidirectional CI scenarios, it is distinguished between infected and uninfected individuals. Then, it is

$$i_3 = \begin{cases} 0 & \text{for uninfected} \\ 1 & \text{for infected} \end{cases}.$$

Before secondary contact starts, one population consists of individuals of genotype $AAbb$, whereas organisms of the other population are all of genotype $aaBB$. In bidirectional CI models, the Ab-population is infected by *Wolbachia* strain W_0, while every individual of the aB-population harbors *Wolbachia* strain W_1. In unidirectional CI models, initially either the island or the mainland population carries infection. Since we assume complete *Wolbachia* transmission, each individual of the infected population is infected.
The individuals' lifecycle starts with migration. A fraction m of the island population is replaced by individuals of the mainland population. Thereby,

m is called migration rate. For the bidirectional CI scenario (Fig. 3.1B), the migration step is formalized by

$$p_{\vec{i}}^{+} = \begin{cases} (1-m)p_{\vec{i}} + m & \text{if } i_1 = 0 \ \wedge \ i_2 = 1 \ \wedge \ i_3 = 1 \\ (1-m)p_{\vec{i}} & \text{else} \end{cases}, \tag{3.1}$$

where $p_{\vec{i}}^{+}$ denotes frequencies of certain geno-/cytotypes after the migration step. The formalization also holds for a unidirectional CI model with infected mainland. If the island is infected and receives uninfected immigrants, equation 3.1 is altered by the value of i_3 and becomes

$$p_{\vec{i}}^{+} = \begin{cases} (1-m)p_{\vec{i}} + m & \text{if } i_1 = 0 \ \wedge \ i_2 = 1 \ \wedge \ i_3 = 0 \\ (1-m)p_{\vec{i}} & \text{else} \end{cases}.$$

After migration local viability selection takes place. In the environment in which the new allele A evolved, this is positively selected. More precisely, on the island an individual has a $1+\frac{1}{2}s$ higher probability to survive if it carries one allele A and it survives $1+s$ times more often carrying two alleles A. With the following function

$$S(i,s) = \begin{cases} 1+s & \text{if } i = 1 \\ 1+\frac{1}{2}s & \text{if } i = 2 \\ 1 & else \end{cases}, \tag{3.2}$$

frequencies $p_{\vec{i}}^{*}$ after selection are obtained by

$$p_{\vec{i}}^{*} = \frac{p_{\vec{i}}^{+} S_A(i_1, s)}{W^*} \tag{3.3}$$

with $W^* = \sum_{\vec{i}} p_{\vec{i}}^{+} S_A(i_1, s)$.
In the reproduction step we consider the inheritance of alleles, transmission of *Wolbachia*, possible fecundity reductions of infected females (only in unidirectional CI models) and hybrid lethality caused by nuclear or cytoplasmic incompatibility. Regarding the inheritance of alleles we assume full recombination, i.e. parents transmit each of their alleles to 50% of their offspring, so that each individual inherits one allele at each locus from each parent. This is formalized by the following weighting factor I, assuming that i denotes the offsprings' and k and l the parents' genotypes. Since both loci are autosomal, inheritance of loci functions equivalently from mother and father to offspring:

$$I(i,k,l) = \begin{cases} 1 & \text{for } i = l = k \wedge i \neq 2 \\ 1 & \text{for } i = 2 \wedge k + l = 1 \\ 0.5 & \text{for } (k = 2 \vee l = 2) \wedge i = k \vee i = l \\ 0.25 & \text{for } k = l = 2 \ \wedge i \neq 2 \\ 0 & \text{else} \end{cases}.$$

Nuclear incompatibilities occur between A and B. Following Turelli and Orr (1995), individuals homogeneous for both incompatible alleles $AABB$ have the highest fitness defect and a proportion l_{NI} is lethal. Other individuals carrying both incompatible alleles $aAbB$, $AAbB$ and $aABB$ suffer less and a fraction hl_{NI} of them dies at embryonal stage, while all other genotypes are not affected and reach adult stage:

$$NI(i,j,l_{\mathrm{NI}},h) = \begin{cases} 1-l_{\mathrm{NI}} & \text{for } i=j=1 \\ 1-hl_{\mathrm{NI}} & \text{for } i\neq 0 \wedge j \neq 0 \\ 1 & \text{else} \end{cases} .$$

The parameters used are called the NI level l_{NI} and the dominance level h. Regarding the dominance level, we will refer to recessive NI if $h = 0$, dominant NI if $h = 1$ and codominant NI if $h = 0.5$.
We describe the expression of CI and transmission of *Wolbachia* by one factor. CI means the lethality of a fraction l_{CI} of the offspring that is caused by different infection types of mating partners. The transmission of *Wolbachia* is strictly maternal, i.e. offspring is infected by the same *Wolbachia* strain as the mother or, if the mother is uninfected, progeny is as well. By defining the following weighting factors, it is assumed that i denotes the offsprings' infection status, whereas k and l are those of mother and father. For models considering symmetric bidirectional CI, both *Wolbachia* strains induce the same CI level. Number of offspring is reduced by the CI level l_{CI} in matings between partners carrying different strains:

$$CI_{Bi}(i,k,l,l_{\mathrm{CI}}) = \begin{cases} 1 & \text{for } i=k=l \\ 1-l_{\mathrm{CI}} & \text{for } i=k \wedge k \neq l \\ 0 & \text{else} \end{cases} . \tag{3.4}$$

The factor (3.4) is altered in order to describe asymmetric CI. Now it is allowed that different strains induce different CI levels. *Wolbachia* strain W_0 induces a CI level $l_{\mathrm{CI},0}$ and correspondingly $l_{\mathrm{CI},1}$ is the CI level expressed by strain W_1. When a W_0-infected male mates with a W_1-infected female, a reduced number $1-l_{\mathrm{CI},0}$ of offspring is produced. Reversely, W_1-infected males produce reduced number $1-l_{\mathrm{CI},1}$ of progeny with W_0-infected females.

$$CI_{ABi}(i,k,l,l_{\mathrm{CI},0},l_{\mathrm{CI},1}) = \begin{cases} 1 & \text{for } i=k=l \\ 1-l_{\mathrm{CI},0} & \text{for } i=k=1 \wedge l=0 \\ 1-l_{\mathrm{CI},1} & \text{for } i=k=0 \wedge l=1 \\ 0 & \text{else} \end{cases} .$$

Unidirectional CI scenarios incorporate a fecundity cost to infected females. This is required to obtain stable infection polymorphisms (see 2.3.2). It is

assumed that infected females only produce a proportion $1-f<1$ offspring compared to uninfected females:

$$CI_{Uni}(i,k,l,l_{\mathrm{CI}},f)=\begin{cases}1 & \text{for } i=k=l=0\\ 1-l_{\mathrm{CI}} & \text{for } i=0\wedge k=0\wedge l=1\\ 1-f & \text{for } i=k=1\\ 0 & \text{else}\end{cases}.$$

Finally, with the next formula

$$p_{\vec{i}}^{-}=\sum_{\vec{k},\vec{l}}p_{\vec{k}}^{*}p_{\vec{l}}^{*}NI(i_1,i_2,l_{\mathrm{NI}},h)I(i_1,k_1,l_1)I(i_2,k_2,l_2)CI_{Bi}(i_3,k_3,l_3,l_{\mathrm{CI}}) \quad (3.5)$$

the frequencies of subsequent generations can be described by

$$p'_{\vec{i}}=\frac{p_{\vec{i}}^{-}}{\sum_{\vec{j}}p_{\vec{j}}^{-}}. \quad (3.6)$$

For models considering asymmetric bidirectional or unidirectional CI the factor CI_{Bi} is to be replaced by CI_{ABi} or CI_{Uni}, respectively.
In order to investigate model dynamics, equilibrium frequencies for various geno- and cytotypes have to be determined, thus $p_{\vec{i}}=p'_{\vec{i}}$ has to be solved. Due to the complexity of this model, analytical derivations of (equilibrium) frequencies are in general impossible to obtain. Therefore, computer programs simulating the model dynamics, i.e. frequency of certain individual types for subsequent generations, were implemented. To obtain equilibrium frequencies the following termination condition was defined: if the sum over differences between the certain geno- cytotype combinations goes below 10^{-10}, the dynamics are defined to be in equilibrium. Termination conditions defined in other studies are of the same magnitude (Servedio and Kirkpatrick, 1997, Telschow et al., 2005a). This equilibrium frequency was disturbed and simulations were started anew to guarantee stability of the equilibrium. Because mainly conditions under which different alleles as well as different infection types coexist were investigated, we considered an allele or cytotype as extinct when its frequency falls below 0.1%. Equivalently, an allele or cytotype was supposed to have reached fixation when exceeding a frequency of 99.9%.

3.3 Results

3.3.1 Without *Wolbachia*: The Classical Dobzhansky-Muller Model

We begin our analysis with the classical Dobzhansky-Muller model without *Wolbachia* infections. This is necessary to later evaluate the impact of CI on stability of genetic divergence. Before secondary contact is established, the island population consists of individuals of genotype $AAbb$. On the mainland, individuals can be characterized by their genotype $aaBB$. Since there is no migration onto the mainland, genetic composition in the mainland population will remain unchanged. With the beginning of secondary contact, the island population receives migrants from the mainland. Whether resident alleles can coexist with migrant alleles a and B or A and b become extinct depends on the parameter values of the migration rate (m), the selection coefficient (s) and on the degree and architecture of reproductive isolation (l_{NI}, h).

Basically, we can determine three different outcomes (Fig. 3.2): (i) without local selection, A and b become extinct for any values of the migration rate; (ii) without NI, equilibrium frequencies of A decrease continuously to zero with increasing migration rate while b becomes extinct for any amount of migrants; (iii) only if both NI level and selection coefficient are positive, coexistence of all four alleles is possible if migration is below a certain threshold value of the migration rate. There is one special case where nuclear divergence is maintained in the absence of local selection, which is for perfect reproductive isolation ($l_{\mathrm{NI}} = 1$ and $h = 1$). Because there occur only two genotypes that produce no offspring with each other, this case is equivalent to a scenario with two perfectly incompatible *Wolbachia* strains which was analyzed by Telschow et al. (2005b) (see section 2.3.2). Then, both genyotypes (or cytotypes) stably coexist up to a critical migration rate of $m_c = 0.172$. In the following, models with incomplete NI will be examined. Since analytical

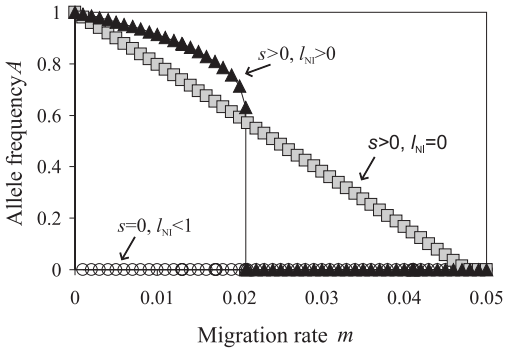

Figure 3.2: Equilibrium frequencies of allele A. Shown are frequencies of allele A as functions of the migration rate. Circles describe a scenario without local selection ($s = 0$). Dynamics with local selection ($s = 0.1$) but without nuclear incompatibilities are shown by boxes. Triangles describe a scenario where both, local selection ($s = 0.1$) and nuclear incompatibilities ($l_{\mathrm{NI}} = 0.5$, $h = 1$) act.

solutions are not obtained, simplified model varations will be discussed first.
(i) Without local selection. Let us first consider the model without local selection. In concordance with Gavrilets (1997) we found that as long as reproductive isolation is imperfect, a and B spread on the island while A and b go to extinction. Although there is an isolating barrier against B, a is not affected by hybrid incompatibilities and replaces A. Thereafter, since B is only deleterious co-occurring with A, the isolating barrier is lost and B goes to fixation as well.
(ii) Without NI. Let us now regard the model with local selection but without nuclear incompatibilities. In this case, equilibrium frequencies can be determined analytically. Let p_A describe the frequency of allele A in the island population. Frequencies of A for subsequent generations can be described by the difference equation

$$p'_A = \frac{(p_A + \frac{1}{2}sp_A^2 + \frac{1}{2}sp_A)(1-m)}{1 + sp_A(1-m)}. \tag{3.7}$$

To determine the equilibrium frequency p_A^* of A, we solve $p_A^* = p'_A = p_A$. This yields

$$p_A^* = 1 - \frac{2m}{s(1-m)}. \tag{3.8}$$

For $p_A^* = 0$ we obtain a threshold value of the migration rate above which A becomes extinct, which is

$$p_A^* = 0 \Longleftrightarrow m_c = \frac{s}{2+s}. \tag{3.9}$$

Therefore, if $s > 0$, equilibrium frequencies of A decrease continuously with increasing migration rate until A has become extinct. For $s = 0$, the only equilibrium frequency is $p_A = 0$, i.e. A becomes replaced by a for every amount of migrants. Since b is not locally selected, the only equilibrium frequency is $p_b^* = 0$. Thus, b becomes replaced by B for any value of the migration rate or selection coefficient.
(iii) With local selection and NI. Only if both, local selection and nuclear incompatibilities act in the model, coexistence of all four alleles is possible as long as migration is sufficiently low. The threshold value of the migration rate below which genetic divergence is maintained is used as a measure for stability of the isolating mechanism (Spirito and Sampogna, 1995, Telschow et al., 2005b). In general, this critical migration describes the migration rate below which all four alleles coexist and above which the resident alleles become extinct. However, for certain parameter ranges dynamics do not show such significant change: for instance it is possible that resident alleles a and

B become extinct for different values of the migration rate. Such cases are discussed in Appendix A. In the following, we will assume that parameter values allow using the term critical migration rate to describe the value at which dynamics switch from the stable coexistence of four alleles to an equilibrium state where resident alleles have become extinct.

Impact of degree of reproductive isolation on critical migration rates. In general, critical migration rates increase with increasing strength of reproductive isolation. Figure 3.3A shows that critical migration rates increase with increasing dominance level. In conclusion, dominant incompatibilities exist up to higher critical migration rates than codominant and recessive NI (Fig. 3.3C). Figure 3.3B contrasts the effect of dominance on complete ($l_{\mathrm{NI}} = 1$) and incomplete ($l_{\mathrm{NI}} = 0.5$) NI. Whereas for small h critical migration rates do not differ much, difference increases with increasing dominance level. This is because for almost perfect reproductive isolation, dynamics diverge to the case in which no hybrids occur ($l_{\mathrm{NI}} = 1$ and $h = 1$). Still, the difference between complete dominant incompatibilities ($h = 1$) and almost complete dominance ($h = 0.99$) is significant when NI is perfect (Fig. 3.3D). In the first case, individuals of the two subpopulations produce no hybrids with each other. Even without local selection, individuals of subpopulations stably coexist up to high critical migration rates. This changes for an almost complete dominance level of $h = 0.99$, where genetic divergence is not maintained without local selection (Figure 3.3D). Already 1% surviving hybrids allow the flow and spread of immigrant alleles in the island population.

Impact of local selection on critical migration rates. Figure 3.3D illustrates the influence of the strength of local selection on the stability of genetic divergence. Critical migration rates increase with increasing selection coefficients. This follows intuitively. Only residents, not migrants, benefit from local selection. Thus, it requires more genetic influx from the mainland to substitute the island genotype by the mainland genotype. This holds independently of the degree of reproductive isolation. Also if no nuclear incompatibilities are involved, the threshold value for the extinction of resident allele A is an increasing function of the selection coefficient s (see equation 3.9). The impact of local selection increases with incrasing strength of NI (Fig. 3.3D) and is thus particularly strong for almost perfect NI (i.e. $h = 0.99$ and $l_{\mathrm{NI}} = 1$). Without local selection, genetic divergence would collapse with the beginning of secondary contact (for $h < 1$). Positive selection on the island leads to a stable coexistence of all four alleles and therefore the maintenance of NI. Since critical migration rates with increasing NI and dominance levels, the difference of critical migration rates in scenarios with strong NI and local selection compared to the model without selection is

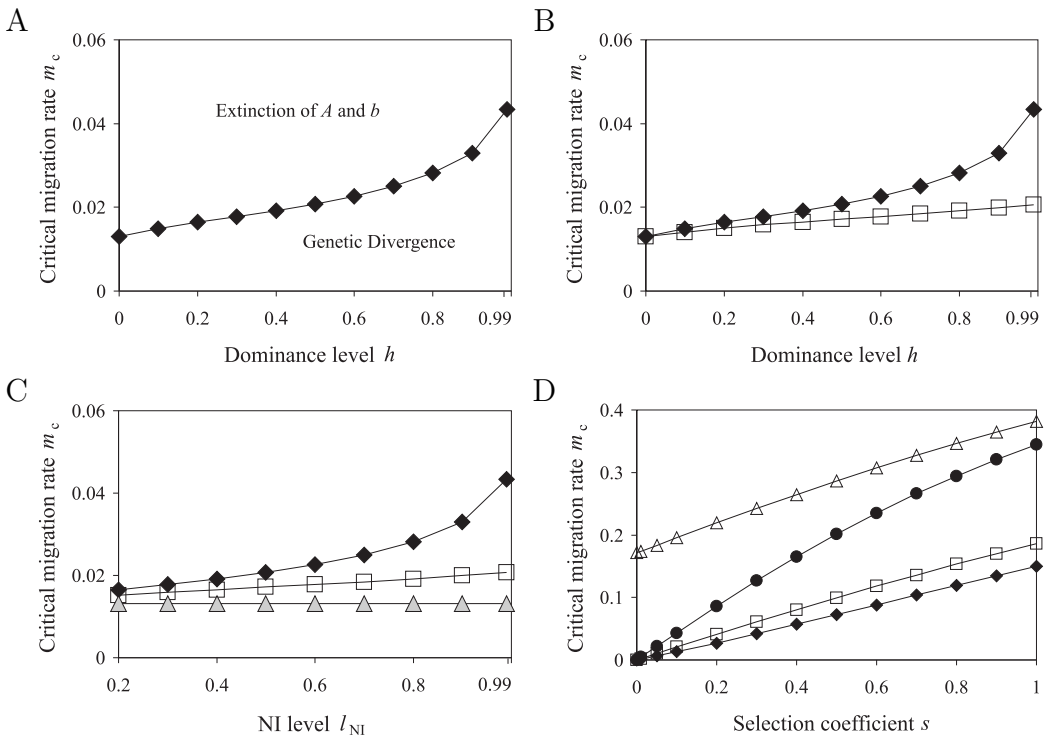

Figure 3.3: Stability of genetic divergence increases with local selection and degree of reproductive isolation. Graph A shows critical migration rates for perfect NI level as a function of the dominance level h. Parameters are $l_{\mathrm{NI}} = 1$ and $s = 0.1$. Graph B shows critical migration rates as functions of the dominance level h for different NI levels $l_{\mathrm{NI}} = 1$ (diamonds) and $l_{\mathrm{NI}} = 0.5$ (boxes). Other parameter is $s = 0.1$. Graph C shows critical migration rates as functions of the NI level for $h = 0$ (triangles), $h = 0.5$ (boxes) and $h = 1$ (diamonds). Further it is $s = 0.1$. Graph D shows critical migration rates as functions of the selection coefficient for different dominance levels $h = 0$ (diamonds), $h = 0.5$ (boxes), $h = 0.99$ (circles) and $h = 1$ (triangles). Further it is $l_{\mathrm{NI}} = 1$.

large. In contrast, local selection has less impact when NI is weak. Still, since genetic divergence is lost without local selection but stably maintained with local selection, dynamics can show significant differences in dynamics depending on the selection coefficient.
In summary, we state that nuclear incompatibilities in the Dobzhansky-Muller collapse unless local selection acts or reproductive isolation is perfect. Then, nuclear divergence can stably be maintained if migration is below a certain critical migration rate. This critical migration increases with strength of local selection and degree of reproductive isolation. Dominant NI can therefore be maintained up to higher migration rates than codominant and recessive NI.

3.3.2 Effect of Bidirectional CI

Now we incorporate *Wolbachia* infections and examine the influence of CI on the stability of genetic divergence. At first, the classical Dobzhansky-Muller model is extended by *Wolbachia* infections causing bidirectional CI (Fig. 3.1B). Besides hybrid fitness defects caused by incompatible alleles, a certain proportion of offspring from matings between partners carrying different *Wolbachia* strains is inviable. Population dynamics in a model considering *Wolbachia* infections only have been analyzed (Telschow et al., 2005b) (see section 2.3.2). Here, the impact of *Wolbachia* on the maintenance of nuclear incompatibilities and potential synergistic effects will be investigated.
In general, we can state that in models without local selection where nuclear divergence is not maintained, *Wolbachia*-induced CI does not enable the four alleles to stably coexist. Although *Wolbachia* delay the time to extinction of resident alleles, the extension by *Wolbachia* does not lead to qualitively different equilibrium states (i.e. maintenance of genetic divergence). In models with local selection where NI is maintained in the absence of bacteria, *Wolbachia* generally leads to an increase of the critical migration rates for NI. This stabilizing effect occurs when critical migration rates for CI are higher than those for NI in the particular models considering only one isolating mechanism.

Wolbachia delay extinction of residents

In the classical Dobzhansky-Muller model it takes 69 generation after secondary contact starts until a has replaced A and 274 for B to replace b. Adding $Wolbachia$ with an incompatibility level of 50% elongates this time span to 178 or 760 generations, respectively. With a strong CI level of 90%, it even takes 1063 or 5064 generations until migrant alleles have replaced the residents alleles. The $Wolbachia$ infection does not enable the stable coexistence of four alleles, but elongates the time span until genetic divergence collapses. Only if CI is perfect ($l_{\mathrm{CI}} = 1$), one genotype is perfectly linked to one $Wolbachia$ strain, reproductive isolation is complete and both genotypes can coexist up to a ceratin critical migration rate below which infection polymorphism is maintained.

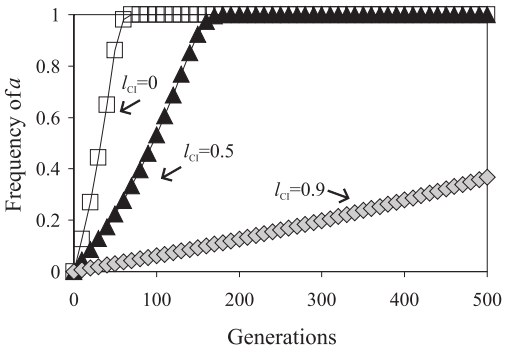

Figure 3.4: Shown are frequencies of allele a as functions of generations after secondary contact starts without $Wolbachia$ (boxes) and with $Wolbachia$ for $l_{\mathrm{CI}} = 0.5$ (triangles) and $l_{\mathrm{CI}} = 0.9$ (diamonds). Further it is $m = 0.03$, $h = 1$ and $l_{\mathrm{NI}} = 0.5$.

Wolbachia increase stability of NI

In models with local selection, genetic incompatibilities are maintained up to a certain critical migration rate. Adding *Wolbachia* generally leads to an increase of this critical migration rate. To investigate interactions of nuclear and cytoplasmic incompatibilities, we begin by considering equilibrium frequencies of allele A in the extended model with *Wolbachia*. Figure 3.5A shows that the threshold migration rates above which A becomes extinct are higher in the presence of *Wolbachia*. This is because *Wolbachia* induce a further isolating mechanism, reduce gene flow and thus increase the degree of reproductive isolation. This graph shows the effect of weak CI levels (10-20%). For higher or even perfect CI, the stabilizing effect is much stronger and nuclear incompatibilities can be maintained up to high migration rates (Fig. 3.5B). For example, 80% CI can result in an elevated critical migration rate of 8.9% (compared to 1.3% without *Wolbachia*). If CI is perfect, no hybrids survive and the genotypes are perfectly linked to one *Wolbachia* strain. In this case, critical migration rates are at least 17.2% (without local selection) and higher when local selection is included. The behavior illustrated by Figure 3.5B is characteristic for a wide parameter range. Independent of the

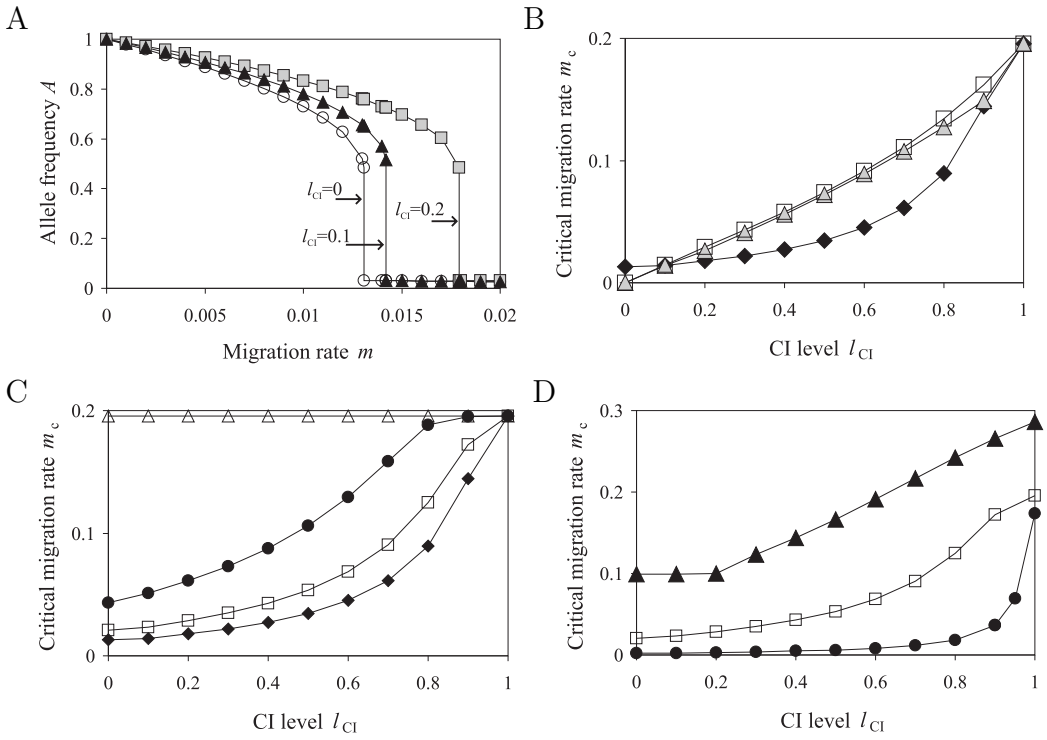

Figure 3.5: Bidirectional CI increases stability of nuclear divergence. Graph A shows equilibrium frequencies of allele A as functions of the migration rate. Circles describe a scenario without *Wolbachia*. Other curves are allele frequencies in the extended model when *Wolbachia* induce weak CI: $l_{\mathrm{CI}} = 0.1$ (diamonds) and $l_{\mathrm{CI}} = 0.2$ (boxes). Other parameters are $l_{\mathrm{NI}} = 1$, $h = 0$ and $s = 0.1$. Graph B shows critical migration rates for coexistence of two *Wolbachia* strains in the presence (triangles) and absence (boxes) of NI. Diamonds describe critical migration rates for nuclear divergence. Parameters are $l_{\mathrm{NI}} = 1$, $h = 0$ and $s = 0.1$. Graph C shows critical migration rates for nuclear divergence as functions of the CI level for $h = 1$ (triangles), $h = 0.99$ (circles), $h = 0.5$ (boxes) $h = 0$ (diamonds) NI. Other parameters are $l_{\mathrm{NI}} = 1$ and $s = 0.1$. Graph D shows critical migration rates for nuclear divergence for different selection strengths $s = 0.01$ (circles), $s = 0.1$ (boxes) and s=0.5 (triangles) as functions of the CI level. Other parameters are $h = 0.5$ and $l_{\mathrm{NI}} = 1$.

strength of NI (as long as $l_{\mathrm{NI}} < 1$ or $h < 1$ holds), adding *Wolbachia* results in an increase of the critical migration rate for nuclear divergence. The original critical migration rate for NI in the model without *Wolbachia* increases in the extended model with increasing CI level up to 19.6% for perfect CI (and $s = 0.1$). Figure 3.5C contrasts the effects of dominance on this stabilizing effect. When NI is perfect and completely dominant ($h = 1$ and $l_{\mathrm{NI}} = 1$), CI has no effect on the critical migration rates for nuclear divergence. This is because all F_1 hybrids die due to perfect NI, thus *Wolbachia* cannot induce an additional isolation barrier. For almost complete dominant NI ($h = 0.99$ and $l_{\mathrm{NI}} = 1$), the stabilizing effect equals that for recessive or codominant NI. Differences arise depending on the value of the critical migration rate for NI in the absence of *Wolbachia*. Since critical migration rates increase up to the same value for perfect CI independent of the dominance level, the relative stabilizing effect is strongest for recessive incompatibilities. For example, stability of recessive NI ($h = 0$) increases from $m_c = 0.013$ in the absence of *Wolbachia* up to $m_c = 0.196$ for perfect CI but from $m_c = 0.043$ up to $m_c = 0.196$ for almost dominant NI ($h = 0.99$). This is because recessive NI alleles migrate well because their negative effects occur only in homozygotes, at the earliest in the F_2 generation. In contrast, CI acts in the first hybrid generation and is thus much more effective at reducing gene flow. In contrast, for a dominance level of $h = 0.99$, genetic incompatibilities alone can strongly impede gene flow by causing lethality of a large proportion of F_1 hybrids.
Figure 3.5D shows stability increase of nuclear divergence for varying strengths of local selection. For strong local selection, critical migration rates for nuclear incompatibilities only increase for higher CI levels. This is because for lower CI levels, genetic incompatibilities are maintained up to higher migration rates than CI and can thus not be stabilized by infection polymorphism.

3.3.3 Effect of Asymmetric CI

Regarding bidirectional CI, we further investigated a more general model allowing different *Wolbachia* strains to induce different CI levels. Asymmetries in the CI level generally diminish critical migration rates for the coexistence of *Wolbachia* strains. Actual values of the critical migration rate depend on which *Wolbachia* strain, resident or migrant, expresses the stronger CI level. In mainland-island models, critical migration rates increase with increasing CI level $l_{\mathrm{CI},0}$ of the resident strain and decrease with increasing CI level $l_{\mathrm{CI},1}$ of immigrants. This is because residing *Wolbachia* W_0 suffer less if the invading *Wolbachia* strain W_1 expresses low CI. On the other hand, migrant

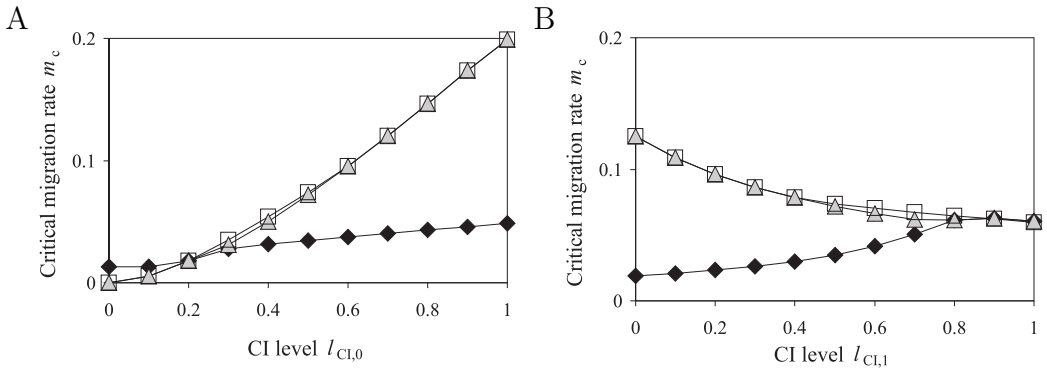

Figure 3.6: Interactions of asymmetric bidirectional CI and NI. In both graphs, the diamonds describe the critical migration rates for genetic divergence as functions of the CI level. The other curves illustrate the critical migration rates for the coexistence of two *Wolbachia* strains in the presence (triangles) and absence (boxes) of NI. Critical migration rates are functions of one CI level only whereas the other CI level is constant. In graph A the migrants' CI level ($l_{\mathrm{CI},1} = 0.5$) and in graph B the residents' CI level ($l_{\mathrm{CI},0} = 0.5$) is held constant. Further parameters are $h = 0$, $l_{\mathrm{NI}} = 1$ and $s = 0.1$.

Wolbachia benefit from their own strong CI level (Telschow et al., 2005b). Figure 3.6 illustrates the interactions of NI and CI. Similar to symmetric bidirectional CI, it can be stated that asymmetric CI can increase the stability of genetic incompatibilities. In contrast, critical migration rates for infection polymorphism are hardly affected by the presence of NI. Since critical migration rates for asymmetric CI are generally lower than those for symmetric CI, the stabilizing effect is weaker than that caused by symmetric CI. Still, for chosen parameter values in Figure 3.6, critical migration rates for nuclear divergence can increase from 1.3% to 4.9% for complete CI level (Fig. 3.6A) or from 1.9% up to 6.1% (Fig. 3.6B) with increasing CI level of the residing or migrating *Wolbachia* strain. This stabilizing effect can turn out weaker or stronger depending on the critical migration rates for CI. In general, high CI levels, especially of the residing strain, cause strong stabilizing effects. If the resident's CI level is weak, migrants can easily invade the island. If the infection polymorphism then collapses for low migration rates, enhancement of NI is either weak or does not occur at all because infection polymorphism is lost for lower migration rates than nuclear divergence.

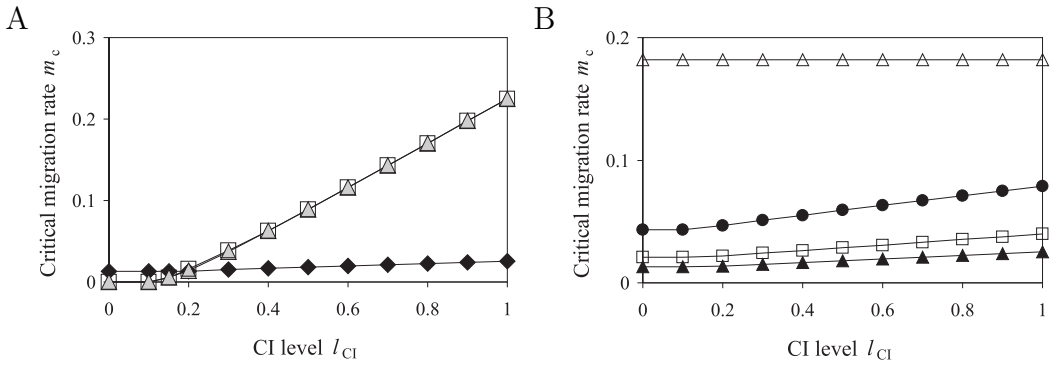

Figure 3.7: Unidirectional CI stabilizes nuclear divergence. Illustrated are interactions of NI and unidirectional CI in a model with infected island. Figure A shows critical migration rates as functions of the CI level for genetic (diamonds) and cytoplasmic divergence interacting with NI (triangles) and in the absence of NI (boxes). Parameters are $f = 0.9$, $l_{\mathrm{NI}} = 1$, $h = 0$ and $s = 0.1$. Graph B shows stability increase of nuclear divergence for different dominance levels $h = 0$ (black triangles), $h = 0.5$ (boxes), $h = 0.99$ (circles), and $h = 1$ (white triangles). Other parameters are $f = 0.9$, $l_{\mathrm{NI}} = 1$ and $s = 0.1$.

3.3.4 Effect of Unidirectional CI

Finally, we consider a model in which only one population is infected by *Wolbachia*. Flor et al. (2007) found that under certain conditions infection polymorphisms between parapatric populations can be maintained, when for instance infected females are less fecund than uninfected females (see 2.3.2). For a wide parameter range, however, unidirectional CI is only maintained up to small critical migration rates, often smaller than those for genetic incompatibilities. For these parameter sets, the *Wolbachia* infection cannot cause stabilization of genetic divergence since the infection polymorphism collapses for lower migration rates than genetic divergence. However, under certain conditions interactions of *Wolbachia*-induced unidirectional CI and genetic incompatibilities can result in a stabilization of single or both isolating mechanisms.

Infected island. Let us first consider a mainland-island model with an infected island. The critical migration rates for cytoplasmic divergence describe the threshold value above which uninfected migrants spread on the island while *Wolbachia* become extinct. These values are generally very high and can exceed 20% for high CI levels. Figure 3.7 shows interactions of NI and CI for such a scenario (note that critical migration rates for *Wolbachia* infection polymorphism are only positive if $l_{\mathrm{CI}} > f$ (Flor et al., 2007)). For intermediate selection strength ($s = 0.1$), CI is hardly affected by nuclear incompatibilities (Fig. 3.7A). On the contrary, critical migration rates for nuclear divergence increase. Since unidirectional CI is maintained up to high critical migration rates, it provides an additional isolating mechanism

over a broad parameter range and thus reinforces NI. Figure 3.7B shows that nuclear divergence increases in stability similarly over a broad range of conditions and differs only for perfect reproductive isolation by NI. Perfect NI does not allow any hybrid production and critical migration rates for cytoplasmic and nuclear divergence are equal for all CI levels. That critical migration rates remain constant for low CI levels for $h < 1$ is because infection polymorphisms already collapse for lower migration rates than genetic divergence. Of course, CI cannot reinforce NI in these cases. On the contrary, nuclear incompatibilities can stabilize infection polymorphism within this parameter range. This generally happens when CI is weak and local selection strong, i.e. when nuclear divergence is maintained up to higher critical migration rates than the *Wolbachia* infection polymorphism.

Infected mainland. Now let us assume that the mainland is infected. Usually, a *Wolbachia* infection easily spreads on the island upon secondary contact, so that critical migration rates are generally low and decrease with increasing CI level. Genetic incompatibilities can strengthen the barrier against invading infected individuals and thus increase stability of infection polymorphisms. Whether NI provide an efficient barrier against *Wolbachia* to spread on the island will depend on the stability of NI alone, which is determined by the strength of local selection and the degree of reproductive isolation. As a consequence of this, the potential reinforcement effect of NI on CI is also primarily determined by the value of the selection coefficient, the dominance and NI level (Fig. 3.9B). Strong local selection as well as strong NI can significantly stabilize infection polymorphism, whereas this effect is much weaker if the strength of local selection, NI and dominance levels are low. On the other hand, stability of nuclear incompatibilities can be reinforced by CI presumed that critical migration rates for infection polymorphism are higher than for nuclear divergence. This generally applies for weak local selection. Then, critical migration rates for genetic divergence are small and can be exceeded by slightly higher critical migration rates for infection polymorphism (Fig. 3.8). Figure 3.9A compares the stability increase of nuclear divergence at different dominance levels. For recessive

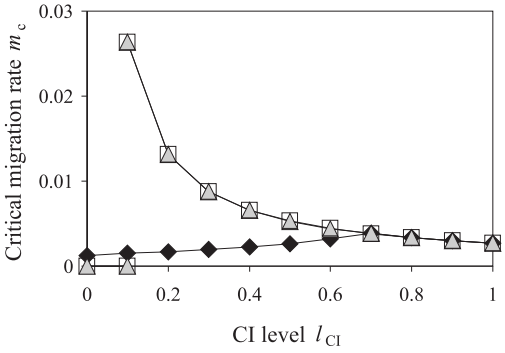

Figure 3.8: Interaction of NI and CI. Shown are critical migration rates as functions of the CI level for genetic (diamonds) and cytoplasmic divergence interacting with NI (triangles) and in the absence of NI (boxes). Other parameters are $s = 0.01$, $h = 0$ and $l_{NI} = 1$.

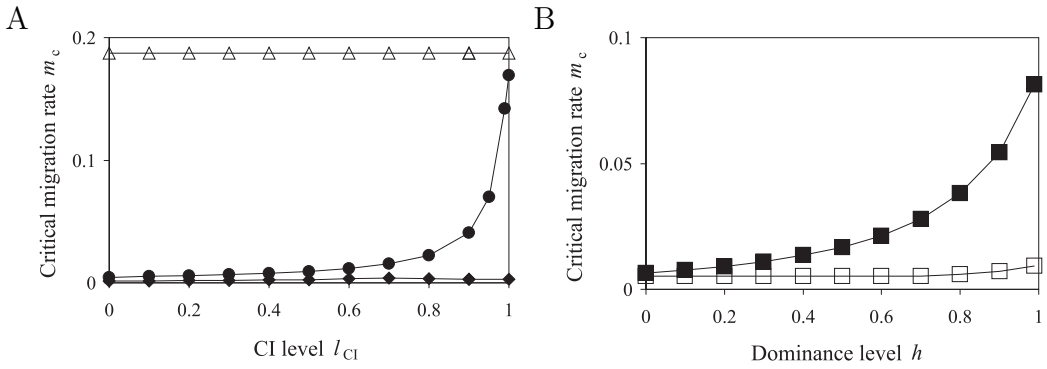

Figure 3.9: Synergy effect of unidirectional CI and NI. Shown are interactions of NI and unidirectional CI in a model with infected mainland. Graph A shows critical migration rates for nuclear divergence as functions of the CI level for different dominance levels $h = 0$ (diamonds), $h = 0.99$ (circles) and $h = 1$ (triangles). Other parameters are $s = 0.01$ and $l_{\mathrm{NI}} = 1$. Graph B shows critical migration rates for infection polymorphism as functions of the dominance level. Parameters are $l_{\mathrm{NI}} = 1$, $l_{\mathrm{CI}} = 0.5$ and $s = 0.1$ (black) and $s = 0.01$ (white).

NI, this effect is comparably weak, but critical migration rates still increase from $m_c = 0.0013$ up to $m_c = 0.0027$. For codominant NI the increase from $m_c = 0.0021$ to $m_c = 0.0079$ is stronger (not shown), but for almost perfect NI ($h = 0.99$), critical migration rates significantly increase from $m_c = 0.0044$ up to $m_c = 0.169$. In this scenario, both isolating mechanisms interact synergistically so that both are significantly stabilized in contrast to models considering only one isolating mechanism, where it is $m_c = 0.0044$ for NI and $m_c = 0.0027$ for CI. When NI and CI act simultaneously, hybrid production is almost prevented so that different genotypes linked to the particular infection status can coexist up to high migration rates.
To summarize, we state that also unidirectional CI can select for Dobzhansky-Muller incompatibilities. In mainland-island models with infected island, nuclear divergence is stabilized over a broad range of conditions. Critical migration rates for infection polymorphism can also be elevated when CI is weak and local selection strong. In models with an infected mainland, CI and NI can act in synergy if NI is strong enough to build up a barrier against infected immigrants.

In conclusion, the results show that *Wolbachia* can have a strong stabilizing effect on nuclear hybrid zones, especially when they induce bidirectional CI and CI levels are high. In principle, the isolating mechanism that is maintained up to a higher critical migration rate causes an elevation of the critical migration rate of the other isolating mechanism. Generally, critical migration rates for bidirectional CI are higher than those for NI. Therefore, *Wolbachia*-induced bidirectional CI can reinforce NI over a broad parameter

range. In contrast, critical migration rates for NI often exceed those for unidirectional CI. In this case, the stability of infection polymorphisms can be increased and infected and uninfected individuals can coexist up to higher migration rates than without NI causing hybrid lethality. In particular, for some parameter values, both isolating mechanisms act synergistically and are significantly more stable when interacting with each other.

3.4 Discussion

In this chapter, the stability of hybrid zones formed after the Dobzhansky-Muller model extended by *Wolbachia*-induced cytoplasmic mating incompatibility was investigated. The main results are that (i) nuclear divergence is not maintained unless local selection favors deleterious allels, (ii) dominant incompatibilities are more robust in the face of migration than recessive incompatibilities and (iii) *Wolbachia*-induced CI increases stability of nuclear incompatibilities over a broad range of conditions.
Our model differs from many previous studies in three ways that will be discussed in turn: diploid genetic architecture, effects of local selection and interaction of two different isolating mechanisms, nuclear incompatibilities and *Wolbachia*-induced cytoplasmic incompatibility.
Diploid modeling. A diploid genetic model was designed. Therefore, the model is more applicable to the genetic systems of most animals than haploid models, which are sometimes employed to reduce computational complexity. Using a diploid model permits us to investigate the effects of dominance of genetic incompatibilities on the stability of genetic divergence under different conditions. This is important because we have shown that the degree of dominance of incompatible alleles has crucial effects. Critical migration rates increase with increasing dominance level, so that dominant incompatibilities are maintained over a broader parameter range than codominant or recessive incompatibilities. This is because dominant incompatibilities can strongly reduce gene flow by affecting F_1 hybrids (see 2.2.5). When incompatibilities are recessive, both incompatible alleles can occur in one individual without causing fitness reductions. Hybrid breakdown only occurs in the F_2 generation resulting in significant gene flow of incompatible loci from the other divergent population. Our results emphasize the importance of diploid modeling. Not only is genetic divergence only possible for lower migration rates, but in a diploid model without local selection nuclear diversity is not maintained at all. Only if NI and dominance are perfect, genetic diversity is stably maintained in the absence of local selection. However, slight changes in the dominance level ($h = 0.99$) result in the collapse of nuclear divergence.

Local Selection. We incorporated the effect of local selection in addition to epistatic selection against hybrids. Thus, fitness of individuals depends on epistatic selection against hybrids that acts regardless of location on the one hand, and also on environmental factors through local selection on the other hand. A study of the Dobzhansky-Muller model by Gavrilets (1997) showed that genetic diversity is not maintained in a two-population model. This model only considered epistatic selection against hybrids, but no local effects. Our results show that within the framework of the typical Dobzhansky-Muller model genetic diversity can be maintained as long as a balance between local selection and migration holds. Thus, local selection is necessary to maintain genetic diversity in the Dobzhansky-Muller model. This changes if incompatibilities are symmetric, i.e. in addition to A and B, also a and b are incompatible. In the latter case, genetic differences are also maintained in the absence of local selection (see Appendix B). The study by Spirito and Sampogna (1995) determined very high critical migration rates for such symmetric incompatibilities. However, they also chose very high values of selection coefficents which resulted in higher stability of genetic divergence. Moreover, it is generally assumed that nuclear incompatibilities occur asymmetrically (Orr, 1995, Turelli and Orr, 2000) so that the the model by Spirito and Sampogna (1995) might represent a less realistic scenario than our model. The original Dobzhansky-Muller model makes no restrictions about the way new alleles evolved. Our work implies that evolution by positive selection might be more stable than, for example, evolution by drift. Cirumstances under which neutral or negatively selected alleles would have evolved seem to be unstable regarding subsequent forming of hybrid zones during secondary contact. Indeed, there is evidence from experimental studies that speciation genes have spread by positive selection (Presgraves et al., 2003, Ting et al., 1998) which is supported by our theoretical investigations. Consequently, the evolution of neutral or negatively selected alleles would play a less important role in parapatric speciation because they are less likely to be maintained when brought into secondary contact.

Two isolating mechanisms. Most previous studies on the role of postzygotic isolation mechanisms in speciation considered only one isolating mechanism. We investigated the interactions of two different isolating mechanisms, nuclear and *Wolbachia*-induced cytoplasmic incompatibilities. *Wolbachia*'s influence on genetic divergence has been investigated theoretically (Telschow et al., 2002). These studies assign an important role to bidirectional CI in preventing gene flow between populations. Our model differs because nuclear loci themselves form part of an isolating mechanism. We found that in the presence of *Wolbachia* the genetic factors causing hybrid dysfunctions are reinforced. In general, it is assumed that alleles causing incompatibilities

are recessive. Analytical approximations of effective migration rates indicate that recessive incompatibilities hardly reduce gene flow (Gavrilets, 1997). Recessive alleles, only unmasked in the F_2 hybrid generation, easily migrate through populations. This readily leads to a collapse of genetic divergence. In contrast, dominant NI efficiently reduces gene flow and is also maintained up to higher migration rates than recessive NI. Therefore, the effect of *Wolbachia*-induced CI on NI is comparably strong for recessive incompatibilities. This is particularly important since most incompatible alleles are supposed to be recessive and implies that *Wolbachia* infections can generally play an important role in maintaining nuclear divergence. Especially, this is true if *Wolbachia* induce strong CI, since the stabilizing effect becomes stronger with increasing CI level. CI levels cover a wide range, from fairly weak CI levels in *Drosophila* (Hoffmann et al., 1994) to complete CI in *Nasonia* (Breeuwer and Werren, 1990). Our model suggests that even weak CI levels can increase the robustness of genetic divergence substantially. Furthermore, asymmetries between CI levels do not prevent this stabilizing effect. In general, the reinforcement of genetic incompatibilities by *Wolbachia*-induced CI should therefore be a common phenomenon in symmetric and asymmetric bidirectional CI scenarios. This stabilizing effect was observed within a broad parameter range. Obviously, the importance of the role of *Wolbachia* depends on the incidence of parapatric populations expressing bidirectional CI. While there is both empirical and theoretical evidence that bidirectional CI might promote speciation (Breeuwer and Werren, 1990, Telschow et al., 2005a), it is argued that it occurs too rarely to be considered an important factor in the evolution of insects (or arthropods) (Hurst and Schilthuizen, 1998). Among insect species, however, there is a growing number of such cases: bidirectional CI has been reported in *Nasonia* (Breeuwer and Werren, 1990), in *Drosophila simulans* (O'Neill and Karr, 1990), in a beetle *Chelymorpha alternans* (Keller et al., 2004), and in several mosquito species (Sinkins et al., 2005, Yen and Barr, 1973) and in a non-insect spider mite species (Gotoh et al., 2005). Further studies of natural populations will have to specify the frequency of naturally occurring bidirectional CI scenarios.

A general problem often addressed in speciation theory is the identification of the speciation-initiating isolating mechanism. Regarding *Wolbachia*-induced CI, it is argued that *Wolbachia* can only play a role if they predate nuclear incompatibilities. In *Nasonia* it was shown that CI precedes other incompatibilities and would thus be the initiating, responsable isolating mechanism (Bordenstein and Drapeau, 2001a). However, this is generally difficult to determine in retrospect. Moreover, it is conceivable that speciation processes are driven by several factors (Werren, 1997; 1998, Shoemaker et al., 1999, Rincon et al., 2006). Especially within the framework of the Dobzhansky-

Muller model populations can diverge genetically and become infected by *Wolbachia* in allopatry. During secondary contact, both would contribute to reproductive isolation, simultaneously and not successively.
A difficult question is whether nuclear incompatibilities caused by only two loci can conspicuously affect hybrid fitness. It has been assumed that hybrid dysfunctions are the consequence of many incompatible loci, each contributing a small effect on sterility or inviability (Orr, 1992). Genetic analyses of postzygotic isolation between closely related species showed that the number of genes involved in incompatibilities ranged from 2 to 190 (Coyne and Orr, 2004). Thereby, a similar historic question arises: were all incompatible genes necessary for the speciation event or were two incompatible genes sufficient to initiate the speciation processes? In this case, further genes expressing incompatibilities could have evolved afterwards substantiating reproductive isolation. It is clear, however, that so called speciation genes exist (Brideau et al., 2006, Presgraves et al., 2003, Ting et al., 1998) and that they alone can cause severe hybrid incompatibilities. However, most research on speciation genes has been conducted on *Drosophila*. Further research is required before general statements about the number of genes involved in evolution of reproductive isolation can be made in *Drosophila* but even more so in other insect or arthropod species. Our results have shown that in the presence of *Wolbachia*, genetic divergence due to weak (recessive) two-loci NI can stably be maintained. Without *Wolbachia*, genetic divergence would be lost more easily. As a result, speciation processes of weakly genetically diverged populations can forcefully be promoted by *Wolbachia*.
Besides bidirectional CI, unidirectional CI is supposed to occur frequently in nature. While the stabilization of genetic divergence by bidirectional CI was observed under a wide range of conditions, unidirectional CI stabilizes genetic diversity only under more restricted conditions, i.e. when unidirectional CI is maintained up to higher migration rates than NI. This is usually the case when uninfected individuals migrate into an infected population or, when infected individuals migrate, local selection is weak. In these cases, however, critical migration rates for genetic divergence can still be up to four times as high as in scenarios without CI. In contrast, nuclear incompatibilities can stabilize infection polymorphisms as well. The critical migration rates for nuclear divergence strongly depend on the strength of local selection. When investigating interactions with CI, we predominantly assumed a selection strength of $s = 0.1$. This is consistent with other theoretical models (Telschow et al., 2005a, Servedio and Kirkpatrick, 1997). (Note that the stabilizing effect of bidirectional CI occurs almost independent of the strength of selection as long $s > 0$). Unidirectional CI could be stabilized by NI for certain values of the selection coefficient: In models with an infected main-

land, we obtained stronger stabilizing effects for low selection coefficients. This is because for weak selection NI are only maintained up to migration rates that lie below those for unidirectional CI. Thus, unidirectional CI is the stronger isolating mechanism and can increase the stability of nuclear divergence. For equivalent models with infected island, critical migration rates for cytoplasmic divergence are generally higher. In these cases, stabilization of one isolating mechanism took place only for rather strong local selection. Contrasting uni- and bidirectional CI, we can state that interactions of NI and unidirectional CI can lead to stabilizing effects as well, but these are not as strong as those in bidirectional CI scenarios and occur across a smaller parameter range. Further, perfect NI can maintain infection polymorphism up to high migration rates in all, unidicretional, symmetric and asymmetric bidirectional CI scenarios. This is clear because no hybrids survive and infection status is perfectly linked to one subpopulation.

Our work also has important implications for reinforcement scenarios, i.e. the evolution of female mating preferences. Females can show assortative mating behavior in order to avoid males with which they would have lower reproductive success. Offspring reduction caused by nuclear or cytoplasmic incompatibilities can both select for the evolution of female mating preferences (Servedio and Kirkpatrick, 1997, Telschow et al., 2005a). Thereby, preferences spread faster when exchange, thus migration between subpopulations is high and genetic or cytoplasmic differences are stably maintained. Servedio and Kirkpatrick (1997) stated that reinforcement only happens when the isolating mechanism is maintained during secondary contact but did not further investigate under which conditions this applies. Our analysis determines the parameter regions where isolating mechanisms are maintained and reinforcement is thus theoretically possible. The critical migration rate can thus be used as an indicator for potential reinforcement scenarios. Furthermore, the actual value of the critical migration rate has implications on the rate of spread of choosy females: preferences spread faster the higher the exchange (i.e. migration) between populations. High critical migration rates guarantee that genetic or cytoplasmic differences are maintained up to high migration rates which allows rapid spread of mating preferences. Telschow et al. (2005a) compared the influence of cytoplasmic versus nuclear incompatibilities on reinforcement. It was shown that *Wolbachia*-induced bidirectional CI promotes the spread of mating preferences more forcefully than nuclear incompatibilities. This is because cytoplasmic divergence is maintained up to higher critical migration rates than genetic divergence and high migration enhances the spread of mating preferences. However, Telschow et al. (2005a) used a haploid model and obtained significantly higher critical migration rates for nuclear incompatibilities than we did in our model. Our

results suggest that the real difference between critical migration rates for nuclear and cytoplasmic incompatibilities is much larger. As a result, *Wolbachia*-induced bidirectional CI selects for the evolution of female mating preferences even more strongly compared to genetic incompatibilities than assumed before. Moreover, since critical migration rates are generally higher when two isolating mechanisms interact, reinforcement is more likely to take place when not only one, but two or even more isolating mechanisms act in synergy. This implies in particular that female mating preferences evolve more easily in *Wolbachia*-infected species.
That nuclear incompatibilities occur simultaneously with *Wolbachia*-induced CI is very likely: *Wolbachia* infections are common in arthropods but especially in insects and should thus have been present during speciation processes in the majority of species. We have shown that when both isolating mechanisms act together, they reinforce each other over a broad range of conditions. This is particularly true for bidirectonal CI that can significantly increase the stability of nuclear based isolating mechanism over a broad range of conditions. This is especially important for recessive NI, which are supposed to occur more frequently than dominant NI but are relatively unstable regarding forming of hybrid zones. In the presence of CI-*Wolbachia*, however, recessive NI can be stably maintained in hybrid zones. Additionally, unidirectional CI can contribute to the stable maintenance of nuclear divergence. The stabilizing effect is less strong and occurs under more restricted conditions. Furthermore, NI can increase the stability of infection polymorphism and for some parameter regions real synergy effects could be observed, so that both mechanisms are much more stable when co-occurring. In conclusion, we find that *Wolbachia* infections substantially contribute to the sustainment of nuclear divergence and are therefore important factors in arthropod speciation.

Chapter 4

Interactions of Haldane-type Incompatibilities and *Wolbachia*-induced Unidirectional CI

In this chapter we analyze interactions of nuclear incompatibilities obeying Haldane's rule and *Wolbachia*-induced unidirectional CI. The classical Dobzhansky-Muller model is altered in order to express sex-specific nuclear incompatibilities (NI) between populations in concordance with Muller's dominance theory. It will be shown that stability of postzygotic isolation is increased when NI and CI occur simultaneously. In particular, NI can cause lethality of infected females and counteract the transmission of *Wolbachia* in taxa with heterogametic females. As a result, CI can act as a strong postzygotic isolating mechanism in Lepidoptera and enhance the maintenance of Haldane-type NI in turn.

4.1 Introduction

In 1922, JBS Haldane observed that:

"When in the offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous [heterogametic] sex."

To date, these asymmetric hybridizations have been observed in many species throughout numerous animal groups (for an overview see Laurie (1997), Coyne (1992)), suggesting that this phenomenon could be a very general pattern and speciation by postzygotic isolation a common process. The generality of Haldane's rule is further underlined by its common occurrence in taxa in which males are the heterogametic sex as *Drosophila* and mammals, as well as in Lepidoptera and birds with heterogametic females. Also other groups show the phenomenon of Haldane's rule, cases have been reported in reptiles, amphibians and nematodes (Laurie, 1997). Theories attempting to explain this phenomenon have been presented in section 2.2.4. Among these explanation approaches, there is strong evidence for the dominance theory, an extension of the Dobzhansky-Muller model: Assume an ancestral population with genotype $aaxx/aax$, at which $aaxx$ describes the homogametic and aax the heterogametic sex. This population becomes separated into two isolated parts. In each population, a new allele A or X appears and replaces a or x, respectively. When populations restore secondary contact, offspring arising from interspecific matings can suffer from incompatibilities between A and X. If heterogametics of the first species (AAx) mate with homogametics of the second species ($aaXX$), offspring aAX and $aAxX$ is produced. Homogametics $aAxX$ are affected by incompatibilities if A as well as X act fairly dominant. There is, however, theoretical (Orr, 1993, Turelli and Orr, 1995) and experimental (Orr, 1992, True et al., 1996) evidence for genes causing hybrid sterility or inviability to be recessive. In this case, if A is dominant or partly dominant and X recessive, homogametics $aAxX$ remain perfectly fit but incompatibilities between A and X are expressed in heterogametic offspring aAX. It should be noted that the reciprocal cross between individuals $AAxx$ and aaX is compatible, at least F_1 hybrids $aAxX$ and aAx do not show negative fitness effects. This is also applicable to taxa with ZW sex chromosome by simply replacing X-linked locus by a Z-linked locus. For simplicity, we will only use the X-notation in the following.
Numerous cases of Haldane-type incompatibilities have been found in *Drosophila* (Coyne and Orr, 1989) and Lepidoptera species (Presgraves, 2002, Jiggins et al., 2001a, Salazar et al., 2004) and sterility as well as lethality have been observed in both groups. Both *Drosophila* and Lepidoptera are also

known to be frequently infected with intracellular bacteria *Wolbachia*. In particular CI-inducing *Wolbachia* have been found in Lepidoptera (Hiroki et al., 2004, Sakamoto et al., 2005, Sasaki et al., 2005) as well as in *Drosophila* (Hoffmann et al., 1986, Hoffmann and Turelli, 1988, Giordano et al., 1995, Jaenike et al., 2006). Because cytoplasmic incompatibility acts as a postzygotic isolating mechanism, it has received attention as a potential factor in evolutionary processes of insects (Laven, 1959, Breeuwer and Werren, 1990). If *Wolbachia* are capable to perceptibly influence speciation processes of their hosts is still under debate since it is argued that bidirectional CI, being able to forcefully promote speciation processes, occurs too rarely to be generally considered an important factor in arthropod evolution. Unidirectional CI should occur more prevalently because it requires only one population to acquire *Wolbachia* infection speciation (Hurst and Schilthuizen, 1998), but as shown in section 2.3 it is not as influential as bidirectional CI in arthropod speciation processes.
It has often been proposed that speciation occurs not only because of one single isolating mechanism but as the result of two or more mechanisms acting together. Especially in connection with *Wolbachia*, several authors suggest that the co-occurrence of nuclear and cytoplasmic incompatibilities can be a driving force in arthropod speciation processes (Werren, 1997; 1998, Shoemaker et al., 1999, Rincon et al., 2006). This should be in particular the case, when isolating mechanism act complementarily: unidirectional CI affects matings between females of the uninfected and males of the infected populations, and Haldane-type NI reduces offspring in the reciprocal mating (it should be noted that in the classical Dobzhansky-Muller model nuclear incompatibilities affect sons and daughters and act in both inter population matings equally). Intuitively, it is clear that postzygotic isolation is stronger when two mechanisms act and not only one isolating mechanism is involved. This does, however, not necessarily lead to a higher stability of reproductive barriers in the face of gene flow. It is therefore important to quantify the effect of interactions on the stability of isolating mechanisms.
In this chapter we analyze theoretically such scenarios with two different, complementary isolating mechanisms. More precisely, we investigate the stability of sex-specific, Haldane-type nuclear incompatibilities in parapatric host populations and examine the influence of CI-*Wolbachia* if one population acquires infection. The classical Dobzhansky-Muller incompatibilities analyzed in the previous chapter affected both, sons and daughter equally, and occured in both interpopulation matings. In contrast, Haldane-type NI only act in one of the first interpopulation matings and affect either sons or daughters. Also, unidirectional CI occurs only between infected males and uninfected females. When both isolating mechanisms co-occur, two pos-

sibilities arise: CI and NI can affect the same mating or act reciprocally. It is therefore necessary to discuss several variations of the model. We will distinguish between models of taxa with XY and ZW sex determination. Furthermore, models with an infected Ax- or aX-population have to be analyzed separately. Thereby we can contrast model variants where CI and Haldane-type NI act reciprocally or in the same mating and contrast effects of male and female lethality on the stability of the single isolating mechanisms. The critical migration rate will again provide a measure for the stability of NI and CI. We will show that in general, that stability of postzygotic isolation is increased when NI and CI occur in reciprocal matings rather than when occurring in the same mating. This stabilizing effect, however, can turn out to be weak, even if CI and NI act reciprocally. Only when NI causes lethality of infected females and counteracts the transmission of *Wolbachia*, NI and CI can significantly reinforce each other. Since this can only happen in taxa with heterogametic females such as Lepidoptera, our results imply that *Wolbachia*-induced unidirectional CI can have strong impact on speciation processes in species with ZW sex determination. Particularly results suggest that Haldane-type lethality should occur more frequently in Lepidoptera than in *Drosophila* species.

4.2 The Model

Sex-specific incompatibilities obeying Haldane's rule are modeled in concordance with Mullers Dominance theory which is based on the Dobzhansky-Muller model. Thus, the model applied here is very similar to the model of the classical Dobzhansky-Muller model in chapter 3. However, as sex-specific incompatibilities between an autosomal and an X-linked allele are considered, the inheritance of alleles, local selection and epistatic selection against hybrids differ. Furthermore, it has to be distinguished between males and females, or more generally, between the homo- and heterogametic sex. Obviously, modeling the *Wolbachia*-related features is consistent with those in section 3.2.

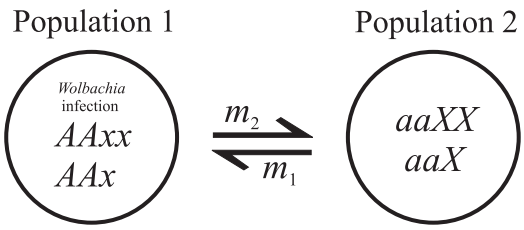

Figure 4.1: Model scenario. A Dobzhansky-Muller model is considered where populations are isolated by sex-specific nuclear incompatibilities and *Wolbachia*-induced CI. With the establishment of secondary contact, interpopulation matings produce less offspring due to nuclear incompatibilities between A and X and *Wolbachia*-induced unidirectional CI.

In the following, we will consider several variations of the model, depend-

ing on sex determination system, migrating behavior or which genotype is initially harboring the *Wolbachia* infection. The concrete model description given below describes the scenario shown by Figure 4.1. Other scenarios are obtained by simple alterations of this description.
Let $p_{\vec{i}}$ and $q_{\vec{i}}$ describe the frequencies of individuals in population 1 and 2, respectively. Thereby, $\vec{i}$ is a 3-dimensional vector at which first two entries i_1, i_2 characterize the genotype and i_3 the cytotype of the different individuals. It is, in accordance with the previous model,

$$i_1 = \begin{cases} 0 & \text{for } aa \\ 1 & \text{for } AA \\ 2 & \text{for } Aa \end{cases},$$

for both, males and females. The second entry i_2 is defined to describe alleles at the X-locus. It has to be noted that homogametics carry two X chromosomes whereas heterogametics carry only one X and one Y chromosome. The Y chromosome will be ignored because it does not have any impact on the population dynamics. We define

$$i_2 = \begin{cases} 0 & \text{for } xx \\ 1 & \text{for } XX \\ 2 & \text{for } Xx \\ 3 & \text{for } x \\ 4 & \text{for } X \end{cases}.$$

Without loss of generality we will only use the notation of the X-locus throughout this chapter. This corresponds to the nomenclature in species with heterogametic males like *Drosophila*. Lepidoptera have sex chromosomes W and Z, females are heterogametic ZW and males homogametic ZZ. To avoid any confusions, we will distinguish between hetero- and homogametics but not use terms as males and females unless the sex determination system was specified. So can the heterogametic sex carrying only one X chromosome either be a (*Drosophila*) male or a (Lepidoptera) female.
The third entry i_3 describes the infection status. Since we will consider unidirectional CI only, it is

$$i_3 = \begin{cases} 0 & \text{for uninfected} \\ 1 & \text{for infected} \end{cases}.$$

Before secondary contact starts, one population consists of Ax-individuals and the other of aX-individuals. Furthermore, we assume that the Ax-population is infected with *Wolbachia*. A mathematical formalization of the starting conditions illustrated in Figure 4.1 is given by

$$p_{\vec{i}} = \begin{cases} 0.5 & \text{if } i_1 = 1 \wedge i_2 = 0 \wedge i_3 = 1 \\ 0.5 & \text{if } i_1 = 1 \wedge i_2 = 3 \wedge i_3 = 1 \\ 0 & \text{else} \end{cases}$$

and

$$q_{\vec{i}} = \begin{cases} 0.5 & \text{if } i_1 = 0 \wedge i_2 = 1 \wedge i_3 = 0 \\ 0.5 & \text{if } i_1 = 0 \wedge i_2 = 4 \wedge i_3 = 0 \\ 0 & \text{else} \end{cases}.$$

Individuals' lifecycle consists of three steps, migration, local viability selection and reproduction. In the migration step, a fraction m_1 of population 1 is replaced by individuals of population 2 and analogously a fraction m_2 of population 2 is substituted by population 1 individuals:

$$p^+_{\vec{i}} = (1 - m_1)p_{\vec{i}} + m_1 q_{\vec{i}},$$

$$q^+_{\vec{i}} = (1 - m_2)q_{\vec{i}} + m_2 p_{\vec{i}}.$$

Note that for $m_1 = 0$ or $m_2 = 0$ the particular mainland-island models are obtained. After migration local viability selection takes place (Table 4.1). In population 1 A is positively selected equivalently as in the original Dobzhansky-Muller model (in section 3.2) and with

$$S_A(i, s) = \begin{cases} 1 + s & \text{if } i = 1 \\ 1 + \frac{1}{2}s & \text{if } i = 2 \\ 1 & \text{else} \end{cases}$$

frequencies after selection in population 1 can be described by

$$p^*_{\vec{i}} = \frac{p^+_{\vec{i}} S_A(i_1, s_A)}{W^*_p}$$

with $W^*_p = \sum_{\vec{i}} p^+_{\vec{i}} S_A(i_1, s_A)$.

In population 2, local selection at the X-locus happens differently. This is because heterogametics carry only one allele at the X-locus. We assume that individuals with one X gain the optimal fitness benefit and survive $1 + s_X$ more often then organisms with an x at the X-locus. Local selection on homogametics is equivalent to local selection at the A-locus. This is formalized by

$$S_X(i, s) = \begin{cases} 1 + s & \text{if } i = 1 \ \vee \ i = 4 \\ 1 + \frac{1}{2}s & \text{if } i = 2 \\ 1 & \text{else} \end{cases}.$$

Local selection on A		Local selection on X			
Both sexes		Homogametics		Heterogametics	
aa	1	xx	1	x	1
aA	$1+\frac{1}{2}s_A$	xX	$1+\frac{1}{2}s_X$		
AA	$1+s_A$	XX	$1+s_X$	X	$1+s_X$

Table 4.1: Local selection acts differently on A and X.

and in population 2 new frequencies are obtained by

$$q_{\vec{i}}^{*} = \frac{q_{\vec{i}}^{+} S_X(i_2, s_X)}{W_q^{*}}$$

with $W_q^{*} = \sum_{\vec{i}} q_{\vec{i}}^{+} S_X(i_2, s_X)$.
In the reproduction step, we consider the inheritance of alleles, transmission of *Wolbachia* and hybrid incompatibilities caused by cytoplasmic or nuclear incompatibilities. Therefore, several weighting factors are defined. Obviously, inheritance of alleles at the A-locus functions as in the original Dobzhansky-Muller model and an individual inherits one allele from each parent. Regarding the X-locus, homogametics also obtain one allele from each parent. Heterogametic offspring inherits its one allele at the X-locus from the homogametic parent. This is because the other sex chromosome ignored would be transmitted by the heterogametic parent to the heterogametic offspring (i.e. in *Drosophila* for example males transmit their Y chromosome to their sons). Assuming that variable i denotes the offsprings' alleles and k and l the parents' alleles, inheritance at both loci is formalized by functions

$$I_A(i,k,l) = \begin{cases} 1 & \text{if } i = l = k \wedge i \neq 2 \\ 1 & \text{if } i = 2 \wedge k + l = 1 \\ 0.5 & \text{if} (k = 2 \vee l = 2) \wedge i = k \vee i = l \\ 0.25 & \text{if } k = l = 2 \wedge i \neq 2 \\ 0 & \text{else} \end{cases}$$

and

$$I_X(i,k,l) = \begin{cases} 0.5 & \text{if } (k + l = 3 \vee (k = 1 \wedge l = 4)) \wedge (i = k \vee i = l) \\ 0.5 & \text{if } (k + l = 4) \wedge (i = 2 \vee i \neq l) \\ 0.25 & \text{if } k = 2 \wedge ((l = 3 \wedge i \neq 1) \vee (l = 4 \wedge i \neq 0)) \\ 0 & \text{else} \end{cases},$$

at which I_A describes inheritance at the A-locus and I_X allele transmission at the X-locus. Nuclear incompatibilities occur between alleles A and X (Table

Table 4.2: Haldane-type nuclear incompatibilities occur between alleles A and X. Thereby, X is assumed to act recessively in order to affect heterogametic F_1 offspring only. Fitness of F_1 hybrids in bold.

	x	X	xx	xX	XX
aa	1	1	1	1	1
aA	**1**	$\mathbf{1 - l_{NI}}$	1	**1**	$1 - l_{NI}$
AA	1	$1 - l_{NI}$	1	1	$1 - l_{NI}$

4.2):

$$NI(i, j, l_{NI}) = \begin{cases} 1 - l_{NI} & \text{if } i \neq 0 \ \wedge \ (j = 1 \ \vee j = 4) \\ 1 & \text{else} \end{cases}.$$

It is supposed that X acts recessively, whereas A is assumed to be fairly dominant. Thereby it is achieved that only the heterogamic suffer from hybrid incompatibilities while homogametic F_1 hybrids $aAxX$ remain viable in concordance with Muller's dominance theory. *Wolbachia*-induced CI is modeled as in section 3.2.1. The function CI_{Uni} describes offspring reduction in matings between infected males and infected females as well as the fecundity reduction of infected females, that produce less (i.e. a proportion $1 - f$) offspring than uninfected females do in compatible matings. Assuming that the cytotypes are denoted by i for the offspring, k for the mother and l for the father, function CI_{Uni} is as follows:

$$CI_{Uni}(i, k, l, l_{CI}, f) = \begin{cases} 1 & \text{for } i = k = l = 0 \\ 1 - l_{CI} & \text{for } i = 0 \wedge k = 0 \wedge l = 1 \\ 1 - f & \text{for } i = k = 1 \\ 0 & \text{else} \end{cases}.$$

The next formula describes the sum over all possible matings multiplied with the particular weighting factors

$$p_{\vec{i}}^{-} = \sum_{\vec{k},\vec{l}} p_{\vec{k}}^{*} p_{\vec{l}}^{*} NI(i_1, i_2, l_{NI}) I_A(i_1, k_1, l_1) I_X(i_2, k_2, l_2) CI_{Uni}(i_3, k_3, l_3). \quad (4.1)$$

By normalizing this sum with the total fitness we obtain the frequencies $p'_{\vec{i}}$ of the next generation

$$p'_{\vec{i}} = \frac{p_{\vec{i}}^{-}}{\sum_{\vec{j}} p_{\vec{j}}^{-}},$$

where $\vec{i}$, $\vec{j}$, $\vec{k}$ and $\vec{l}$ are 3-dimensional vectors with $\vec{i} = (i_1, i_2, i_3)$, $i_1 = 0, 1, 2$ and $i_2 = 0, ..., 4$ and $\vec{j}$ is defined equivalently. As $\vec{k}$ describes the genotype of the homogametic and $\vec{l}$ of the heterogametic parent, it is $\vec{k} = (k_1, k_2, k_3)$, $k_1, k_2 = 0, 1, 2$ and $k_3 = 0, 1$ and $\vec{l} = (l_1, l_2, l_3)$, $l_1 = 0, 1, 2$, $l_2 = 3, 4$ and $l_3 = 0, 1$. *Wolbachia* is always maternally transmitted. Equation 4.1 describes a

model where females are homogametic. A model for taxa with heterogametic females is easily obtained by exchanging indices k_3 and l_3 in the function CI_{Uni}. Then, we obtain dynamcis in a model where the heterogametic sex transmits the *Wolbachia* infection.

4.3 Results

4.3.1 Without *Wolbachia*: Stability of X-Autosomal Incompatibilities

The dynamics of this model with Haldane-type sex-specific incompatibilities are very similar to those of the original Dobzhansky-Muller model with autosomal-autosomal incompatibilities. The Dobzhansky-Muller model has been elaborated in section 3.3. Since many results of both models coincide, Results for Haldane-type NI are briefly presented but differences to the classical model are accentuated.

Without selection $s_A = s_X = 0$

Without local selection, genetic divergence is not maintained. This is true for X-autosomal as well as as for autosomal-autosomal incompatibilities (see section 3.3). For autosomal-autosomal incompatibilities, however, there was one exception: It was shown that genetic divergence can persist in the absence of local selection when reproductive isolation is perfect, i.e. no hybrids are produced. This does not hold for X-autosomal incompatibilities. Even if it is $l_{\mathrm{NI}} = 1$, hybrid incompatibilities only affect heterogametic hybrids (aAX) but not homogametic hybrids $aAxX$ in one interpopulation mating ($AAx \times aaXX$). In the reciprocal mating ($AAxx \times aaX$), all F_1 hybrids aAx and $aAxX$ are perfectly fit. Therefore, interpopulation matings always produce viable hybrids regardless of the NI level. As a result, in mainland-island models migrants spread for $s_A = s_X = 0$ whenever it is $m > 0$. In two-way migrations model, both alleles A and X become extinct and there is a return to the ancestral genotype $aaxx/aax$.

With symmetric selection $s_A = s_X > 0$

In section 3.3 it was shown that autosomal-autosomal incompatibilities are only maintained if there is local selection on the newly evolved alleles. This applies likewise to X-autosomal incompatibilities. In this model two cases of mainland-island models have to be distinguished, depending on whether $aX-$ or Ax-individuals are populating the island. This is because local selection as well as hybrid incompatibilities act differently at the autosomal locus and the X-locus. Table 4.2 shows fitness reduction of NI in different

	Ax-pop migrates	aX-pop migrates	two-way migration
$s = 0.01$	0.0013(0.0013)	0.0019(0.0019)	0.0016(0.0016)
$s = 0.1$	0.0137(0.0136)	0.0195(0.0193)	0.0171(0.017)
$s = 0.2$	0.0283(0.0279)	0.0395(0.0391)	0.0357(0.0354)

Table 4.3: Critical migration rates for Haldane-type nuclear incompatibilities for perfect NI ($l_{\mathrm{NI}} = 1$) (and imperfect NI ($l_{\mathrm{NI}} = 0.9$)). It is $s = s_A = s_X$ in the two-way migration model, $s = s_A$ when the aX-population is the migrating population and $s = s_X$ when Ax-individuals are the migrants.

genotypes. Apparently, allele x is never involved in incompatibilities, i.e. none of the affected hybrids carries an x-allele. This is an advantage for all individuals carrying x und thus for the Ax-population. As a result, critical migration rates are higher if the Ax-population is the island population and receives migrants. Table 4.3 shows critical migration rates for different model scenarios. Since observations of Haldane's rule suggest that nearly all hybrids of the particular sex are sterile or inviable, we assume perfect or nearly perfect NI level. Within this parameter range, critical migration rates slightly differ for perfect ($l_{\mathrm{NI}} = 1$) or imperfect ($l_{\mathrm{NI}} = 0.9$) NI. In contrast, considered values of the selection coefficients lead to much different values of the critical migration rates which increase with increasing strength of local selection (Table 4.3).

With asymmetric migration or selection

For symmetric migration ($m_1 = m_2$), genetic diversity is maintained up to a critical migration rate of $m_{c,sym} = 1.7\%$ (for $s_A = s_X = 0.1$) laying inbetween the critical migration rates for the mainland-island models. These are the perfect asymmetric cases, where it is $m_1 = 0$ or $m_2 = 0$. Depending on the direction in which migration takes place, genetic divergence is mainted up to critical migration rates of $m_{c,1} = 0.0195$ ($m_2 = 0$) or $m_{c,2} = 0.0137$ ($m_1 = 0$), respectively. Figure 4.3 shows asymmetric critical migration rates. Thereby, migration in one direction is constant and the critical amount of migration in the other direction is determined. Critical migration rates $m_{c,2}$ and $m_{c,1}$ increase with increasing number of migrating individuals in the other direction due to a more balanced influence of selection and NI. Since the Ax-population can stand a greater amount of genetic influx, a critical migration rate of $m_{2,crit}$ is determinable for some $m_1 > m_{c,sym}$, whereas in the other direction the system collapses for a certain value of $m_2 < m_{c,sym}$. In general, higher critical migration rates $m_{c,1}$ or $m_{c,2}$ are obtained when for gene flow in the opposite direction it holds $m_2 < m_{c,sym}$ or $m_1 > m_{c,sym}$.

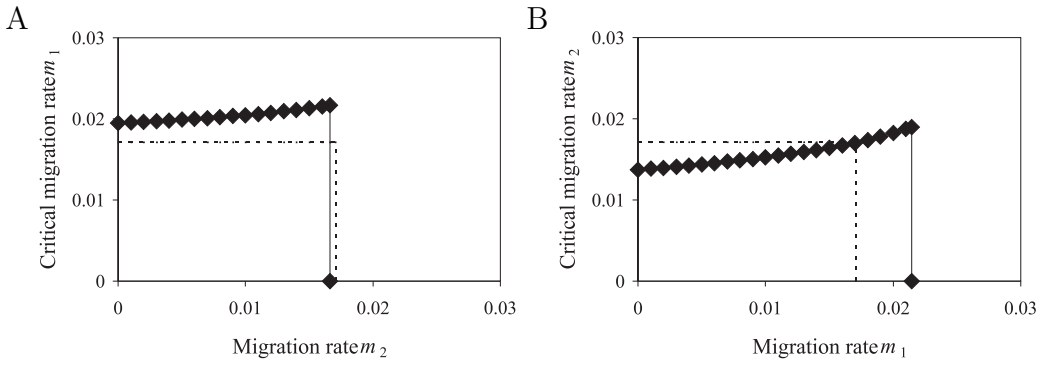

Figure 4.3: Shown are critical migration rates as functions of the other migration rate which is held constant. In graph A gene flow (m_2) is constant from population 1 into population 2. For graph B the reverse case applies. The dotted line denotes the critical migration rate $m_c = 0.017$ for symmetric migration ($m_1 = m_2$). Other parameters are $s_A = s_X = 0.1$ and $l_{\mathrm{NI}} = 1$.

We remark that this is different in the classical Dobzhansky-Muller model. Autosomal-autosomal incompatibilities and local selection act equally in both populations and it was shown that critical migration rates are at maximum when gene flow is symmetric in both directions.

Local selection acts differently on alleles A and X (Table 4.1). Figure 4.2 shows that maximum critical migration rates are obtained for some $s_X > s_A$. In the mainland-island models, Ax-residents can tolerate a higher amount of aX-migrants than reversely for equal values of s_A and s_X. The effective benefit from local selection against genetic influx for the particular populations is therefore equal for some $s_X > s_A$. In conclusion, highest critical migration rates are obtained when s_A takes a value smaller than s_X. This is also a difference to the classical Dobzhansky-Muller model. Since local selection acts equally in both populations, maximum critical migration rates are obtained if selection coefficients take equal values and asymmetries in the selection coefficients destabilize the system.

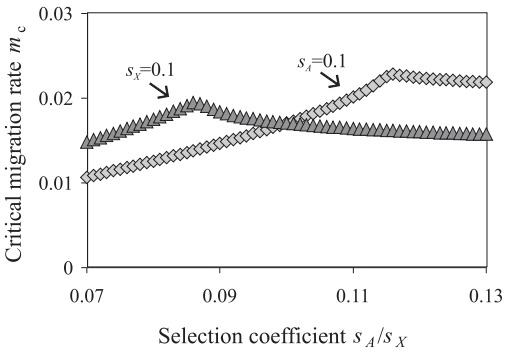

Figure 4.2: Shown are critical migration rates as functions of one selection coefficient while the other is held constant. Triangles describe critical migration rates as function of s_A while $s_X = 0.1$ is held constant. Analogously, diamonds are critical migration rates as a function of s_X while $s_A = 0.1$ is constant. Other parameter is $l_{\mathrm{NI}} = 1$ and migration is symmetric.

4.3.2 Effect of Unidirectional CI

Model scenarios

Our aim is to evaluate the stability of the two interacting isolating mechanisms in different scenarios and to investigate if and how different isolating mechanisms affect each other. Depending on the sex determination system, which genotype is initially linked to the *Wolbachia* infection and whether infection is found in the mainland or island population, dynamics can show significant differences. These differences arise because F_1 hybrid generations are differently affected by isolating mechanisms in the different model variations, i.e. isolating mechanisms can overlap in one mating or complement each other by affecting different matings. Moreover, whether NI cause lethality of infected daughters or sons can be responsible for different model outcomes. Since there are many different scenarios, we give a verbal description of interpopulation matings and effects on the regarding F_1 hybrid generation before presenting results from simulations for the different scenarios.

mating		F_1 hybrids		
		Scenario 1a		
female	male	$aAxX$	aAx	aAX
$AAxx^W$	aaX	$\frac{1}{2}(1-f)^W$	$\frac{1}{2}(1-f)^W$	
$aaXX$	AAx^W	$\frac{1}{2}(1-l_{\mathrm{CI}})$		$\frac{1}{2}(1-l_{\mathrm{NI}})(1-l_{\mathrm{CI}})$
		Scenario 1b		
female	male	$aAxX$	aAx	aAX
AAx^W	$aaXX$	$\frac{1}{2}(1-f)^W$		$\frac{1}{2}(1-f)(1-l_{\mathrm{NI}})^W$
aaX	$AAxx^W$	$\frac{1}{2}(1-l_{\mathrm{CI}})$	$\frac{1}{2}(1-l_{\mathrm{CI}})$	
		Scenario 2a		
female	male	$aAxX$	aAx	aAX
$AAxx$	aaX^W	$\frac{1}{2}(1-l_{\mathrm{CI}})$	$\frac{1}{2}(1-l_{\mathrm{CI}})$	
$aaXX^W$	AAx	$\frac{1}{2}(1-f)^W$		$\frac{1}{2}(1-f)(1-l_{\mathrm{NI}})^W$
		Scenario 2b		
female	male	$aAxX$	aAx	aAX
AAx	$aaXX^W$	$\frac{1}{2}(1-l_{\mathrm{CI}})$		$\frac{1}{2}(1-l_{\mathrm{CI}})(1-l_{\mathrm{NI}})$
aaX^W	$AAxx$	$\frac{1}{2}(1-f)^W$	$\frac{1}{2}(1-f)^W$	

Table 4.4: Mating table for the first hybrid generation produced by interpopulation matings for different model scenarios. Index W marks individuals with a *Wolbachia* infection. Further, f is the fecundity reduction of infected females, l_{CI} the CI level, and l_{NI} the NI level. It should be noted that NI is supposed to be nearly complete, thus $l_{\mathrm{NI}}>0.9$.

Scenario 1: Ax-population infected
(a) Males heterogametic
Interpopulation matings occur between uninfected males aaX and infected females $AAxx$. They produce infected sons aAx and infected daughters $aAxX$. Neither NI nor CI act while in the reciprocal cross between infected males AAx and uninfected females $aaXX$ the number of offspring is reduced due to CI. Sons and daughters emerging from matings between uninfected females $aaXX$ and infected males AAx die due to CI. Moreover, sons aAX are affected by genetic incompatibilities. Both incompatibility types NI and CI affect hybrids produced in the same mating. If CI is strong, interactions with NI should not have strong effect on the dynamics respecting stability of infection polymorphism because male hybrids affected by NI suffer from CI anyway. The other way round, however, an effect on nuclear divergence should be observable. CI expand hybrid incompatibilities to the homogametic sex so that postzygotic isolation between populations is reinforced.

(b) Females heterogametic
Uninfected males $aaXX$ mate with infected females AAx. Infected sons $aAxX$ and daughters aAX are produced, but incompatibilites between A and X results in a reduction of number of daughters. The reciprocal cross, between infected males $AAxx$ and uninfected females aaX, is incompatible because of CI. Surviving offspring is uninfected and consists to equal parts of sons $aAxX$ and daughters aAx. As illustrated in Table 4.4, nuclear incompatibilities affect infected daughters and counteract the *Wolbachia* transmission. In contrast to the first scenario, NI and CI do not overlap but act in reciprocal matings. Thus, both incompatibility types complement each other, i.e. we expect that stability of postzygotic isolation is increased more strongly than in scenario 1a and particularly, NI should impede the spread of *Wolbachia*.

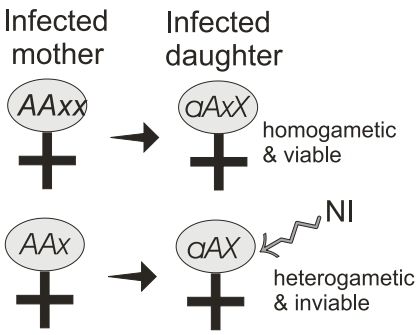

Figure 4.4: This graph illustrates the influence of the sex determination in scenario 1. In scenario 1a, females are homogametic and infected mothers produce viable, infected daughters. In scenario 1b, females are heterogametic, infected daughters are inviable and cannot pass on the *Wolbachia* infection.

Scenario 2: aX-population infected
(a) Males heterogametic
When uninfected males AAx mate with infected females $aaXX$, infected sons aAX suffer from nuclear hybrid incompatibilities, whereas infected daughters $aAxX$ are completely viable. Cytoplasmic incompatibility occurs in the

reciprocal mating between infected males aaX and uninfected females $AAxx$. Also in this case, NI and CI act in different crosses and when acting together the level of postzygotic isolation is expected to be increased. In contrast to scenario 1b, NI causes lethality of infected male progeny. Infected daughters remain viable and can still pass on the *Wolbachia* infection.
*(*b) Females heterogametic
Uninfected males $AAxx$ and infected females aaX produce completely viable and infected sons $aAxX$ and daughters aAx. The other mating occurs between infected males $aaXX$ and uninfected females AAx. Due to CI the number of offspring is reduced. Further, daughters aAX suffer from hybrid dysfunctions. Equivalently to the first scenario (1a), NI and CI act symmetrically, i.e. affect offspring from one mating type only.

Results from simulations

In our analysis we had to distinguish between three different migrating behaviors in each of the model scenarios. That means that 12 variations of the model had to be examined. In the results section, we will focus on a couple of selected scenarios to concentrate on the main findings and on the decisive factors that are responsible for different outcomes in different model variants. In particular, we will start with results from mainland-island models of senario 1a and 1b. Scenario 2 as well as models with two-way migration will be discussed more briefly afterwards.

Interactions of NI and CI - Scenario 1: *Ax*-population infected

*(*a) Males heterogametic

In the mainland-island models there are two cases to be distinguished, depending on which population is infected. If the mainland is infected, infected individuals migrate to the uninfected island. The critical migration rates for *Wolbachia* infection polymorphism generally decrease with increasing CI level, thus they are highest for low CI levels. In the parameter range in which critical migration rates for infection polymorphism are higher than critical migration rates for genetic diversity, nuclear divergence is stabilized when interacting with CI (Fig. 4.5A,B). This mainly applies when local selection is weak, i.e. when genetic divergence is only maintained up to low critical migration rates. *Wolbachia*-induced CI can then stabilize nuclear diversity ($m_c = 0.0013$ up to $m_c = 0.0044$) because CI reinforce the effect of NI by killing female offspring in matings in which otherwise only sons would be inviable due to NI. If NI is maintained up to higher migration rates than CI, cytoplasmic divergence is slightly stabilized (Fig. 4.5A,B). Although NI does not directly influence hybrid fitness when CI is complete, they prevent the migrants' alleles from spreading on the island. Therefore local selection on alleles initially connected to uninfected individuals is maintained and enforces the resistance of uninfected residents. If the island is infected, the infection is generally maintained up to high migration rates. In this case, the stability of infection polymorphism is hardly affected by NI (Fig. 4.5C). On the other hand, CI cause an increase of the critical migration rate for nuclear divergence. Without *Wolbachia* in-

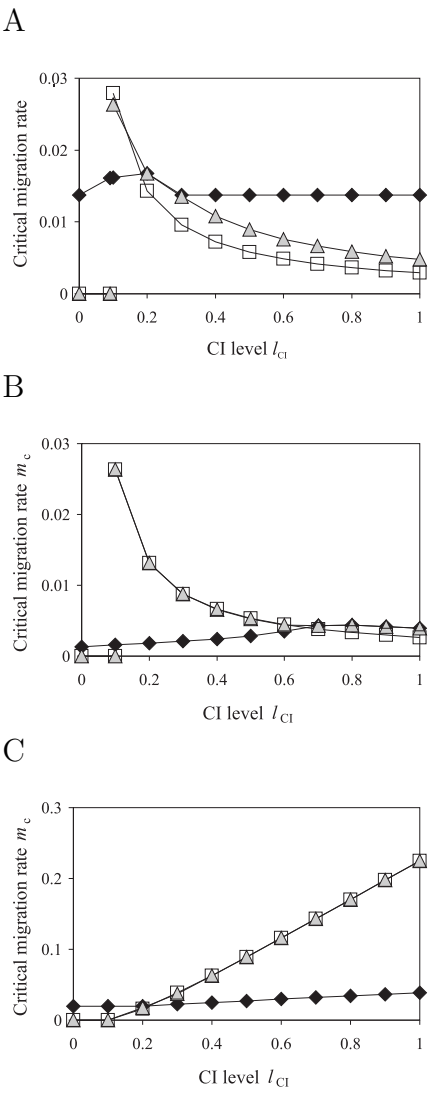

Figure 4.5: Interactions of NI and CI with an infected *Ax*-population and heterogametic males. Shown are critical migration rates as functions of the CI level for Haldane-type incompatibilities (diamonds) and *Wolbachia* infection polymorphisms interacting with NI (trianlges) and without NI (boxes). Figures A and B describe mainland island models with infected mainland for different selection strengths $s_X = 0.1$ in A and $s_X = 0.01$ in B. Other parameters are $l_{\mathrm{NI}} = 1$ and $f = 0.1$. Figure C describes the scenario with an infected island population. Parameters are $l_{\mathrm{NI}} = 1$, $s_A = 0.1$ and $f = 0.1$.

fection, genetic diversity is maintained up to a migration rate of $m_c = 0.0195$. Incorporating a *Wolbachia* infection on the island causes an increase of this critical migration rate up to $m_c = 0.039$ (Fig. 4.5C) Resident alleles can therefore persist the double amount of migrants when infected with *Wolbachia* before going to extinction.

To summarize, we state that in this scenario NI's impact on the stability of CI is weak because NI affects hybrids that are for the most part inviable due to CI. However, NI maintains genetic divergence and thus local selection acts in favor of the connected cytotype so that critical migration rates for infection polymorphism can be elevated. CI affects female hybrids that would not be suffering from NI. In cases in which CI is maintained up to higher critical migration rates than NI, CI reinforces postzygotic isolation and leads to a higher stability of nuclear diversity.

*(*b) Females heterogametic

Now we consider an equivalent scenario but assume that females are heterogametic. In this case, CI and NI affect different hybrids from different crosses. CI occurs in matings between infected males $AAxx$ and uninfected females aaX, whereas NI causes lethality of female offspring produced by uninfected males $aaXX$ and infected females AAx. Since NI kills infected daughters, the transmission of *Wolbachia* is antagonized. Infected sons survive, but they cannot transmit the infection to their offspring. In the model with an infected mainland population, NI thus provides a strong barrier against infected immigrants and infection polymorphism is significantly more stable than in a model without NI (Fig. 4.6). This is especially true for perfect NI ($l_{\mathrm{NI}} = 1$) (Fig. 4.6A) but also holds for imperfect NI ($l_{\mathrm{NI}} = 0.9$) (Fig. 4.6B). Because stability of NI is strongly influenced by the strength of local selection, the barrier against infected immigrants becomes stronger with increasing selection coefficient, but can even be effective for weak local selection (Fig. 4.6C). On the other hand, as CI causes lethality in hybrids that would survive without infection, CI enhances postzygotic isolation so that also nuclear divergence is stabilized. If CI and NI are both complete, male immigrant genes cannot flow through the island population because when mating with females from the island, CI results in the death of common progeny. Female migrants produce viable males only, these are, however, infected and can thus not reproduce with females from the island population either. Thus, both isolating mechanisms together build up a strong barrier against gene influx from the mainland. Dynamics in the model with an infected island population are comparable to those in scenario 1a with het-

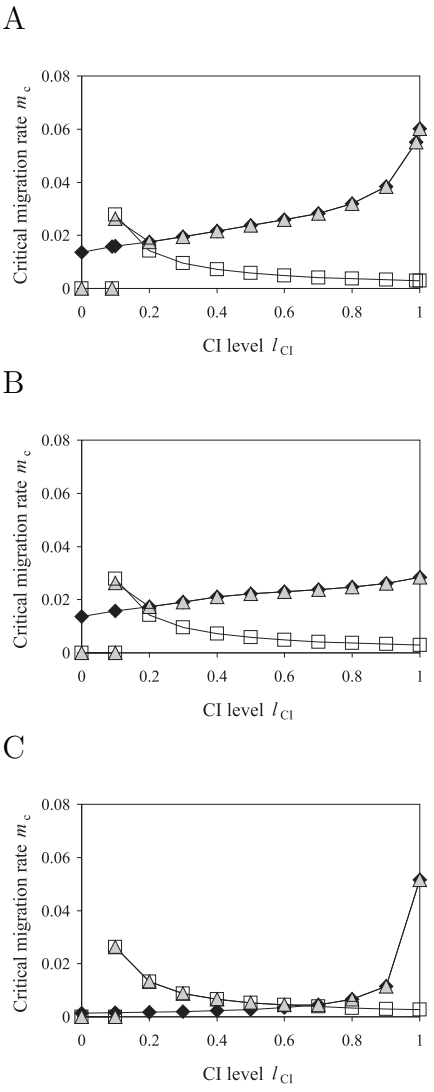

Figure 4.6: Interactions of NI and CI with an infected mainland Ax-population and heterogametic females. Shown are critical migration rates as functions of the CI level for Haldane-type incompatibilities (diamonds) and *Wolbachia* infection polymorphisms interacting with NI (trianlges) and without NI (boxes). Parameters are $l_{\mathrm{NI}} = 1$, $s_A = s_X = 0.1$ and $f = 0.1$ in A, $l_{\mathrm{NI}} = 0.9$, $s_A = s_X = 0.1$ and $f = 0.1$ in B and $l_{\mathrm{NI}} = 1$, $s_A = s_X = 0.01$ and $f = 0.1$ in C.

erogametic males, at least regarding the stability of genetic divergence which is elevated (from $m_c = 0.019$ up to $m_c = 0.039$ for $l_{\mathrm{CI}} = 1$) in the presence of CI-*Wolbachia*. On the other hand, as NI weakens the infected population and impede transmission of *Wolbachia*, critical migration rates for infection polymorphism are slightly reduced (not shown).
To summarize we state that in this model scenario, NI has strong impact on stability of infection polymorphisms by causing lethality of infected females. When the mainland population is infected, the spread of *Wolbachia* on the island is therefore strongly impeded. CI as an isolating mechanism is maintained up to high critical migration rates and can enhance nuclear divergence in turn. In models with infected island, CI is maintained up to high migration rates, enhances postzygotic isolation and causes an increase of critical migration rates for nuclear divergence.

Interactions of NI and CI in Scenario 2: aX-population infected
Let us first consider the model with an infected mainland. Results are similar to those described above for scenario 1a. Significant stabilizing effects as in scenario 1b have not been observed. This is because here nuclear incompatibilities can only cause lethality of immigrating infected males. This can provide a barrier against the infection to spread, but as *Wolbachia* is strictly maternally transmitted this barrier is only weak. Sex determination systems hardly affect dynamics, only small differences can be observed between scenarios 2a and 2b. In scenario 2a, NI and CI occur in reciprocal matings while they affect hybrids of one mating only in scenario 2b. In general, stabilizing effects are stronger in scenario 2a where NI and CI act in reciprocal matings than in 2b. However, reciprocal occurence of NI and CI does not lead to a strong reinforcement of isolating mechanisms (Fig. 4.7). When the island population initially harbors the *Wolbachia* infection, dynamics are also similar to those in the other scenarios (Fig. 4.5C). Critical migration rates for infection polymorphism are hardly altered whereas critical migration rates for nuclear divergence increase with increasing CI level.

Two-way migration
Interactions of CI and NI in the two-way migration models for the different scenarios do not provide further insights into potential stabilizing effects of NI and CI than the mainland-island models. In a model with an infected Ax-population (scenario 1) we can state a strong influence of the sex determination system just like in the mainland-island models. When females are heterogametic and NI causes lethality of infected females, NI and CI both strongly increase in stability. With heterogametic males, the single isolating mechanisms are hardly affected by the interaction with the other. In the sce-

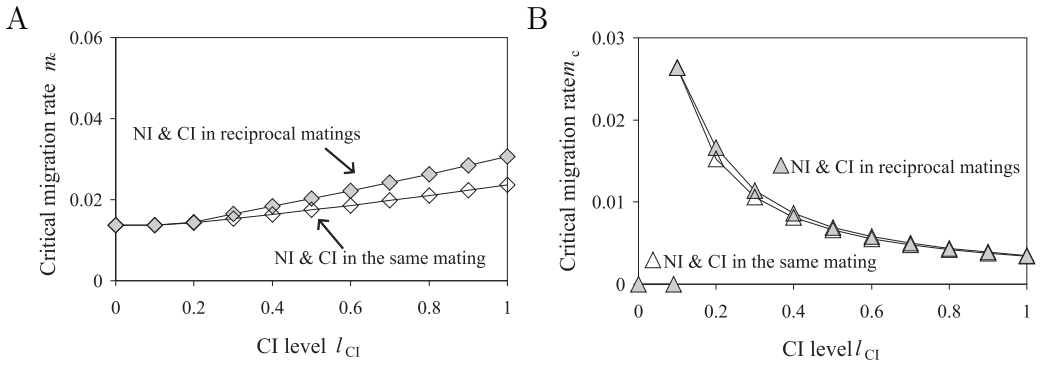

Figure 4.7: Reciprocity alone has no crucial effect on nuclear or cytoplasmic divergence in scenario 2. Gray symbols denote the model variant with heterogametic males where NI and CI act in reciprocal matings. White symbols denote scenarios with heterogametic females where NI and CI affect the same mating. Graph A shows critical migration rates for nuclear divergence in models with infected island for scenarios 2a (gray) and 2b (white). The increase is a stronger if CI and NI act complementarily (gray) than for NI and CI acting in the same mating (white). Graph B shows critical migration rates for infection polymorphism for scenario 2a where NI causes lethality of infected males (gray) and for scenario 2b where NI causes lethality of uninfected females (white). Parameters are $l_{\mathrm{NI}} = 1$, f=0.1, and $s_X = s_A = 0.1$ in both graphs.

narios with an infected aX-population, no significant stabilizing effects were observed either. Whether males or females are heterogametic, i.e. isolating mechanisms happen in the same or reciprocal matings has only weak impact on the outcomes. When males are heterogametic and isolating mechanisms occur in reciprocal matings, critical migration rates for both isolating mechanisms can increase more strongly than in models with heterogametic females (comparable to Fig. 4.7).

In conclusion, we state that interactions of two different isolating mechanisms generally lead to a higher stability of the single mechanisms. This effect is stronger when NI and CI act complementarily. Since CI always reduces the number of uninfected offspring, it is thus required that NI affects infected progeny in order to increase the degree of postzygotic isolation. However, reciprocity alone does not always provoke strong stabilization of postzygotic isolation. This only applies if nuclear incompatibilities cause lethality of infected females and thus counteract the *Wolbachia* transmission, while the elimination of infected males has only weak influence on the spread of *Wolbachia* (Fig. 4.8). Since Haldane-type NI affects heterogametics only, NI and CI as isolating mechanisms can only show this synergy effect when occurring simultaneously in ZW taxa, but not in XY taxa. These results suggest in particular that unidirectional CI might have more impact on speciation processes of ZW taxa and that Haldane's rule for lethality should be more common in Lepidoptera than in *Drosophila* species.

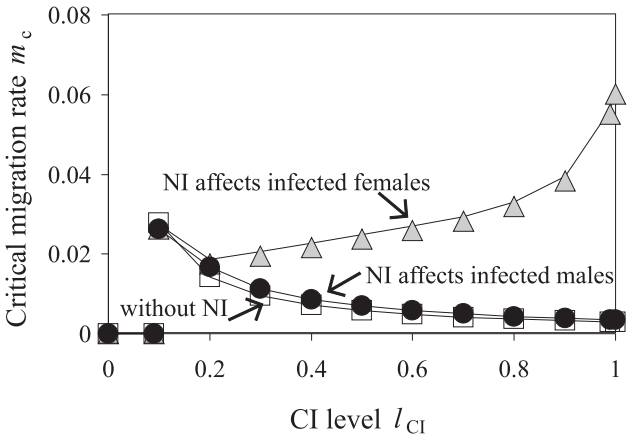

Figure 4.8: Female lethality prevents *Wolbachia* from spreading. Shown are critical migration rates for *Wolbachia* infection polymorphism without NI (boxes) and with NI causing lethality of infected males (scenario 2a, circles) and infected females (scenario 1b, triangles). Migration is unidirectional and the mainland harbors the infection. Other parameters are $l_{\mathrm{NI}} = 1$, f=0.9 and $s_A = s_X$=0.1.

4.4 Discussion

In this chapter we investigated interactions of two postzygotic isolating mechanisms, genetic incompatibilities showing the phenomenon of Haldane's rule and *Wolbachia*-induced unidirectional CI in a diploid two-population model. Our main results are that interactions of NI and CI can lead to an increase of the stability of postzygotic isolating mechanisms. This stabilizing effect is generally stronger when NI and CI act complementarily, i.e. affecting hybrids in reciprocal matings, but is especially strong in taxa with heterogametic females as Lepidoptera, where NI causes lethality of infected females and thus impede the spread of *Wolbachia*. While interacting, NI and CI are both maintained up to higher critical migration rates than in the single models considering either CI or NI. This synergy effect does not occur in taxa with heterogametic males as *Drosophila*, because here Haldane-type NI causes lethality of infected males which cannot effectively prevent *Wolbachia* from spreading.

We focused our theoretical investigations on how *Wolbachia*-induced unidirectional CI influence and interact with sex-specific genetic incompatibilities. We distinguished between different model scenarios, depending on migrating behavior and the sex determination system. Thereby, we could compare the impact of nuclear incompatibilities causing lethality in infected or uninfected males or females. Further, cases in which isolating mechanisms affect the same or reciprocal matings could be contrasted. We have shown that NI and CI need to act complementarily to increase the stability of postzygotic isolation mechanisms. NI is therefore required to occur in matings between infected females and uninfected males, because CI is always expressed in matings between infected males and uninfected females. Being complementary alone, however, is not sufficient to significantly stabilize isolating mecha-

nisms. Dynamics crucially depend on the sex determination system, because this determines whether males or females are affected by NI. Results have shown that NI can effectively create a barrier against the spread of *Wolbachia* when it causes lethality of infected females. In mainland-island models with infected mainland or two-way migration models, a *Wolbachia* infection normally spreads through populations very easily. But when NI kills infected hybrid daughters of invading infected females, the *Wolbachia* infection will remain restricted to the migrants genotype and will not spread through the formerly uninfected population. Hence, CI as an isolating mechanism can persist up to much higher migration rates. In turn, this allows elevation of critical migration rates for nuclear incompatibilities up to the same values and each isolating mechanism is significantly reinforced by the interaction with the other. Remarkably, this stabilizing effect is yielded for different strengths of local selection and also if NI is imperfect, i.e. a small fraction of females carrying incompatible alleles survive. In contrast, lethality of infected sons does not yield the same strong effect, because *Wolbachia* is maternally transmitted and can thus easily spread through viable, infected daughters. As a result, this synergy effect can occur in ZW taxa such as Lepidoptera where heterogametic females are affected by Haldane-type NI, but not in XY taxa such as *Drosophila* where heterogametic males are inviable. Assuming that *Wolbachia* infect two-thirds of insect species, and *Drosophila* and Lepidoptera species in particular, results suggest that Haldane-type lethality should occur more abundantly in ZW taxa because in XY taxa NI collapses more easily. Indeed, Haldane's rule for lethality was observed more frequently in Lepidoptera than in *Drosophila* species (Table 2.2). Our results provide evidence that besides genetic factors also cytoplasmic elements can be responsible for such patterns. However, the same pattern was observed in birds and mammals. Cases of Haldane-type lethality occur more frequently in birds with ZW sex determination system than in mammals with XY sex chromosomes. Of course, *Wolbachia* cannot be the reason for such a pattern outside arthropod species. However, as there is no general explanation for Haldane's rule but at least two or even more that can apply to different patterns, female lethality might be frequent in birds and Lepidoptera for different reasons. That among *Drosophila* species hybrid male sterility occurs more frequently than male lethality is usually explained by faster-male evolution causing sterility and dominance causing sterility and lethality both contribute to Haldane's rule in *Drosophila* (see 2.2.4). The faster-male theory is based on the assumption that insect male genitalia evolve faster than other morphological characters. It is not clear whether an equivalent theory explains why also in mammals Haldane-type sterility occurs more frequently than lethality. Especially, since experimental data for all groups (apart from *Drosophila*) is too

rare to make ultimate statements about the causes and frequencies of different incompatibility types in different taxa. Therefore, besides genetic factors *Wolbachia* should be incorporated in regarding investigations and considered as potential cause for such patterns occurring in insects.
That *Wolbachia* might influence speciation processes of their hosts was first suggested by (Laven, 1959). Within the last decade evidence for CI playing a role in arthropod evolution has increased (Werren, 1998, Telschow et al., 2005a), but the general role of *Wolbachia* in speciation processes is still controversial (Hurst and Schilthuizen, 1998). In contrast to unidirectional CI, there is theoretical (Telschow et al., 2002; 2005a) and empirical (Breeuwer and Werren, 1990) evidence that bidirectional CI can strongly influence speciation processes of host species. Within this chapter we have not shown results for bidirectional CI interacting with sex-specific NI, because these are equivalent to models with autosomal-autosomal incompatibilities and state a significant stability increase of nuclear divergence (chapter 3). Bidirectional CI is thus more effective than unidirectional CI in stabilizing NI, for both autosomal-autosomal and X-autosomal incompatibilities. However, it is argued that bidirectional CI occurs rarely and can therefore not be considered as an important speciation factor (Hurst and Schilthuizen, 1998). In contrast, unidirectional CI is supposed to be a more general pattern because only one population is required to harbor an infection. Due to its frequent occurrence, unidirectional CI could play an important role in insect speciation and many authors have suggested that unidirectional CI interacting with other complementary reproductive isolating mechanisms such as genetic incompatibilities or behavioral isolation could be important in speciation processes of insects (Shoemaker et al., 1999, Werren, 1997; 1998). This is supported by our results. Although real synergy effects were only stated for ZW taxa, stability of postzygotic isolation is generally increased when both mechanisms act simultaneously. Lepidoptera and *Drosophila* are predestined for such studies on interacting isolating mechanisms. Both are known to be frequently infected by *Wolbachia* and many cases of Haldane-type lethality have been reported in both groups, thus it is likely that Haldane-type incompatibilities have co-occurred with *Wolbachia*-induced alterations of the hosts' reproductive system. Of course, our theoretical investigations are not restricted to these two groups, but also apply to other arthropod species with the regarding sex determination systems and possible *Wolbachia* infections. For example, other species belonging to Heteroptera and Orthoptera can express Haldane incompatibilities (Laurie, 1997) and were found to be infected by *Wolbachia* (Werren et al., 1995a).
In this study we focused on analyzing the stability of NI and CI when both interact. It will further be interesting to examine such scenarios with respect to

gene flow. Studies on gene flow between parapatric populations showed that under certain conditions gene flow reduction factors can multiply if different isolating mechanisms act simultaneously (Kobayashi and Telschow, 2008). Both, male inviability (Kobayashi and Telschow, 2008) and unidirectional CI (Telschow et al., 2007) have been shown to reduce gene flow between populations, so that in the combined model gene flow reduction should be stronger than in the single models. In analogy to our analysis, it can be investigated if and how complementariness of isolating mechanisms affects gene flow reduction and whether the sex determination system, i.e. which sex suffers from NI, has an impact. As indicated in the descriptions of the different model scenarios above, we would expect stronger gene flow reduction in scenarios where CI and NI affect reciprocal matings. However, we also expected that stability of postzygotic isolation increases more strongly when NI and CI affect reciprocal matings than when acting in the same mating. Our analysis has shown that reciprocity alone is not the decisive factor regarding stability of isolating mechanisms and did hardly affect stability of postzygotic isolation in scenarios with heterogametic males in particular. The sex determination, i.e. whether male or female hybrids are afflicted by NI, turned out to be more pivotal. An isolating mechanism that systematically prevents collapsing of genetic or cytoplasmic differences can strongly increase the stability of postzygotic isolation. This was observed in ZW taxa when NI cuts off the *Wolbachia* transmission by killing infected females and significant effects on stability of both isolating mechanisms could be stated. How NI and CI affect each other regarding gene flow reduction between parapatric populations, i.e. whether complementariness of isolating mechanisms or the sex of lethal hybrids has more impact on dynamics remains to be elaborated in further studies.

To summarize, we state that unidirectional CI and Haldane-type incompatibilities can reinforce each other when acting simultaneously in reciprocal matings. Particularly, Haldane-type NI can impede the spread of a *Wolbachia* infection by killing infected female offspring. This allows unidirectional CI to persist up to high migration rates. Then unidirectional CI is able to strengthen nuclear divergence in turn. These findings provide strong evidence for unidirectional CI co-occurring with Haldane-type NI playing an important role in speciation processes of arthropods, especially in Lepidoptera or other taxa with a ZW sex determination system. To establish *Wolbachia* as a general promoter of speciation events, it is important to show that bacteria can influence speciation processes under a broad range of conditions. This particularly includes investigations on unidirectional CI because this might be the most frequently occurring manipulation induced by *Wolbachia*. In this chapter we have shown that unidirectional CI can indeed have crucial

impact on host speciation processes. Especially in Lepidoptera, it is possible that *Wolbachia* contributes to the frequent occurence of Haldane-type female lethality.

Chapter 5

How many species are infected with *Wolbachia*?

The incidence of *Wolbachia*, i.e. the number of infected arthropod species has been estimated to be around 20%. This estimate emerged as the result of several *Wolbachia* screenings, where arthropod, mainly insect species were tested for infection. Those tests were mostly performed on only one or two individuals per species. Thereby, low infection densities within species are likely to be overlooked. Depending upon the prevalence, i.e. the infection density within species, the proportion of infected species is supposably significantly higher than 20%. In the following chapter data from several *Wolbachia* screenings will be analyzed within the framework of the beta-binomial model in order to (1) find a distribution of the prevalence and, based on this, (2) derive estimates of the incidence. The main findings are that (1) the prevalence distribution obeys a 'most-or-few' infection pattern in a sense that the *Wolbachia* infection density within one species is typically either very high or very low and (2) the incidence of *Wolbachia* can be estimated to be almost 70%.

5.1 Introduction

For all research fields related to *Wolbachia*, i.e. their impact on evolutionary processes, it is of interest to know how many species are actually infected. A *Wolbachia* infection can be detected by PCR analysis (O'Neill et al., 1992) and since this method became an established and relatively easy method to detect bacteria, numerous arthropods, mainly insects have been sampled to determine the proportion of infected species. Currently the incidence of *Wolbachia* is estimated to be around 20% (Werren et al., 1995a, Werren and Windsor, 2000). This estimate emerged as the result of studies in which few, mostly only one or two individuals per species, were tested for infection. The following problem arises in studies based on a single or few individuals per species. If an individual is infected, the species is rightly classified as infected. One or a few uninfected individuals, however, result in the classification of this species to be uninfected. This method works when infection frequencies within infected populations are always high. However, low infection frequencies are reported as well. For instance, Tagami and Miura (2004) found only 3.1% of the Japanese butterfly *Pieris rapae* to harbor *Wolbachia*. The probability of detecting this infected species would obviously have been low if only a single specimen had been tested. Furthermore, infection levels may depend, in part, on the mode of reproductive manipulation induced by *Wolbachia*; for instance, *Wolbachia* that induce male-killing are expected to occur at lower frequencies (5%-50%) within species than those causing cytoplasmic incompatibility (CI) (Hurst et al., 2000), but there is also theoretical (Turelli, 1994, Flor et al., 2007) and empirical (Hoffmann et al., 1998) evidence that CI-*Wolbachia* infections can occur at intermediate or low frequencies. Since prevalence differs across species, it can be assumed that the $\sim$ 20% infection level found in several studies by testing a few individuals per species is an underestimate. There is another study based on mostly one-individual samples that gives much higher infection rates of 76% (Jeyaprakash and Hoy, 2000). However, this study used a 'long PCR' method that is much more sensitive to trace *Wolbachia* molecules, and therefore environmental contaminants are more likely to be detected. In contrast, most other studies using standard PCR techniques give consistent estimates of infection levels (Table 5.2).

Incidence	The overall proportion of species that harbors *Wolbachia* infections
Prevalence	The proportion of infected individuals within one species

Table 5.1: Definition of incidence and prevalence

	Number of samples	Incidence
Werren and Windsor (2000)	159	18%
Werren et al. (1995a)	139	15%
West et al. (1998)	53	15%
Kikuchi and Fukatsu (2003)	103	31%
Total	**5**22	**2**1%
Jeyaprakash and Hoy (2000)	62	73%

Table 5.2: Proportion of infected species found among one-individual samples from several *Wolbachia*-screenings

In contrast to studies that test very few individuals of many species, there are other surveys that study single species in more detail. The largest sample was taken from the mosquito *Culex pipiens* (Rasgon and Scott, 2003), of which 1090 individuals were tested. In this case, it was clear that these mosquitos harbor *Wolbachia* (Laven, 1959, Yen and Barr, 1973) and Rasgon and Scott (2003) focused on studying infection dynamcis within this species. Also, other surveys might base on prior knowledge. For instance, Jiggins et al. (2001b) tested several species of *Acraea* butterflies. Their aim was to estimate the degree to which previous surveys have underscored the proportion of infected species. Large samples of different *Acraea* species were collected and tested for infection. It was known before that groups of these species are infected, which were excluded from the analysis. However, knowing that *Wolbachia* infections are common in this group might have been the reason to this more detailed survey on *Wolbachia* infection rates. Breeuwer and Jacobs (1996) surveyed different spider mite species for *Wolbachia* infection. At this point, one predatory mite was reported to harbor *Wolbachia*. But there was further evidence that *Wolbachia* infections might be common in mites: Cytological studies reported the presence of intracellular microbes in reproductive tissues. Also embryo mortality and male-biased sex ratios in F_1 offspring indicating the presence of *Wolbachia* were observed before.
Published data from *Wolbachia* screenings reports an increasing frequency of infected species with number of individuals tested (Table 5.3). Obviously, infections, especially low prevalence infections, are more likely to be detected in larger samples. However, the fact that 12 infected species were found among the 13 species of which more than 100 individuals were tested might be rather due to prior evidence of the presence of *Wolbachia* than only to large sample sizes. Also, the average prevalence of *Wolbachia* infections increases with the sample size (Fig. 5.1).

The prevalence averaged over all samples is 22% and increases by excluding one-individual samples, one- and two-individual samples etc. up to 54% when only samples with more than 100 tested individuals are considered. Again, this trend is obviously due to the fact that low prevalence infections are likely to be overlooked by sampling only few individuals. It might, however, also be a result of the fact that among the largely sampled species there are many that were known to be infected before. This does not necessarily mean that these infections are high prevalence infections, but high prevalence infections are more easily detected than low prevalence infections. As a result, high prevalence infected species are more likely to be investigated in detail just because they are found more easily (as *C. pipiens* showing a 99.4% prevalence infection).

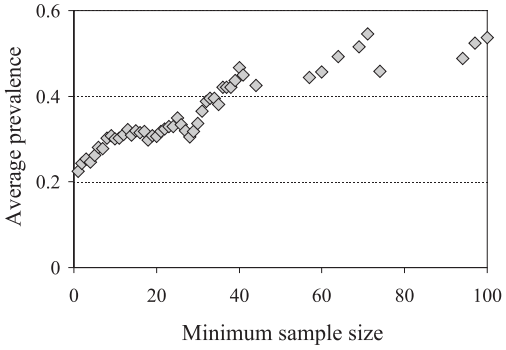

Figure 5.1: This graph shows the average prevalence for data sets with a certain minimum sample size, i.e. complete data, data without one-individual samples, without one- and two- individual samples etc.

To summarize we state that the whole data set contains many one-individual samples from predominantly randomly chosen species on the one hand and fewer large samples from species that were likely known to be infected before on the other hand. First sample type data probably present a random choice of species, but the incidence cannot be estimated without information on the prevalence of the single infected species. Information on the prevalence can be obtained from larger samples, though these might not present a random choice of species.

In the following, we present an analysis of published data from 18 *Wolbachia*-screenings: Bouchon et al. (1998), Jiggins et al. (2001b), Kikuchi and Fukatsu (2003), Kondo et al. (1999), Nirgianaki et al. (2003), Ono et al. (2001), Plantard et al. (1999), Rasgon and Scott (2003), Rokas et al. (2002), Shoemaker et al. (2002; 2003), Tagami and Miura (2004), Thipaksorn et al. (2003), Van Borm et al. (2001), Vavre et al. (2002), Werren et al. (1995a), Werren and Windsor (2000) and West et al. (1998). In total, we summarized and evaluated data from 9113 individuals of 893 species. Data are analyzed within the famework of a beta-binomial model. A function describing the prevalence distribution within species is estimated. Estimates of the total percentage of infected species are provided by this function as well. This estimation procedure requires weighting infection densities with the corre-

Table 5.3: Proportion of infected species found for certain data subsets depending on the number tested per species.

Sample size	Number of samples	Infected species
1	522	21%
2	103	26%
≥ 10	109	53%
>100	13	92%

sponding sample size. Therefore, large samples have crucial impact whereas one-individual samples do not preponderate. This can be a problem because large samples might bias the outcome as tested species were often known to be infected before. Confirming this distortion within large samples, we found that skipping large samples yields more realistic results. By using the whole data set the incidence of *Wolbachia* is likely to be overestimated. Finally we will show that the prevalence of infections follows a 'most or few' infection pattern, i.e. the infection density within one species is typically very high (>90%) or very low (<10%). Based on this prevalence distribution, it can be shown that former incidence estimates of around 20% are underestimations and that more likely 60-70% of species are infected.

5.2 The Beta-Binomial Model

5.2.1 Mathematical Description of the Beta-Binomial Model

Let a random variable X be binomially distributed with $X \sim Binom(q, n)$. Then, the probability for $P(X = k)$ is

$$P(X = k) = f(k, q) = \binom{n}{k} q^k (1 - q)^{(n-k)} \quad k = 0, ..., n.$$

Let us further assume that q is not a constant parameter but a random variable with a beta distribution. Then, the distribution of q is given by

$$p(q) = \frac{1}{B(\alpha, \beta)} q^{\alpha-1} (1 - q)^{\beta-1},$$

where is $B(\alpha, \beta) = \frac{\Gamma(\alpha)\Gamma(\beta)}{\Gamma(\alpha+\beta)}$ and Γ is the conventional Gamma function. The mean and the variance of a beta distribution are

$$E[q] = \frac{\alpha}{\alpha + \beta} \quad \text{and} \quad Var[q] = \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)}.$$

Then we determine the marginal distribution of X given q:

$$P(X=k|q) = P(X=k|\alpha,\beta) = \int_{-\infty}^{\infty} f(k,q)p(q)dq$$

$$= \int_{-\infty}^{\infty} \binom{n}{k} q^k (1-q)^{n-k} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} q^{\alpha-1}(1-q)^{\beta-1} dq$$

$$= \binom{n}{k} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \int_{-\infty}^{\infty} q^{k+\alpha-1}(1-q)^{n-k+\beta-1} dq$$

$$= \binom{n}{k} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \frac{\Gamma(k+\alpha)\Gamma(n-k+\beta)}{\Gamma(k+\alpha+n-k+\beta)}$$

$$\int_{-\infty}^{\infty} \frac{\Gamma(k+\alpha+n-k+\beta)}{\Gamma(k+\alpha)\Gamma(n-k+\beta)} q^{k+\alpha-1}(1-q)^{n-k+\beta-1} dq$$

$$= \binom{n}{k} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \frac{\Gamma(k+\alpha)\Gamma(n-k+\beta)}{\Gamma(k+\alpha+n-k+\beta)} = \binom{n}{k} \frac{B(k+\alpha, n-k+\beta)}{B(\alpha,\beta)}$$

This is a distribution called beta-binomial distribution. Finally, we determine mean and variance of X using iterated mean and variance formulae:

$$E\left[\frac{X}{n}\right] = E\left[E\left[\frac{X}{n}|q\right]\right] = E\left[q\right] = \frac{\alpha}{\alpha+\beta} = \mu \tag{5.1}$$

$$Var\left[\frac{X}{n}\right] = E\left[Var\left[\frac{X}{n}|q\right]\right] + Var\left[E\left[\frac{X}{n}|q\right]\right]$$

$$= E\left[\frac{q(1-q)}{n}|\alpha,\beta\right] + Var\left[q|\alpha,\beta\right]$$

$$= \frac{\alpha\beta}{n(\alpha+\beta)^2} + \frac{n-1}{n}\frac{\alpha\beta}{(\alpha+\beta)^2(\alpha+\beta+1)}$$

$$= \frac{\alpha\beta}{n(\alpha+\beta)^2}\left(1 + \frac{n-1}{\alpha+\beta+1}\right). \tag{5.2}$$

5.2.2 *Wolbachia* Screenings in a Beta-Binomial Model

In this section we demonstrate why the beta-binomial model provides an appropriate framework to analyze the above described data from *Wolbachia* screenings. Let us assume that we test a certain number n_j of individuals of a species (j) for $Wolbachia$ infection. This species has a certain prevalence of infection, q_j, i.e. the proportion of infected individuals within this species. Then the number of individuals tested positive within this sample, X_j, is binomially distributed.

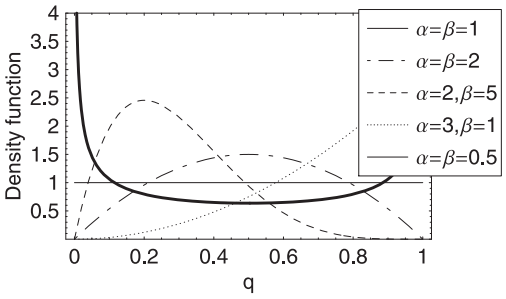

Figure 5.2: This graph shows different forms of a beta distribution depending on parameter values α and β.

The parameters of this binomial distribution are the number of individuals tested (n_j) and the prevalence within this species (q_j), so that $X_j \sim Binom(q_j, n_j)$. The parameter q_j is unknown. To determine this average prevalence within species, one would usually estimate q_j by moment estimators, i.e. the quotient of the number of infected individuals and the number of individuals tested. Here, this method would only work if we considered one species only or all species showed the same prevalence. However, it must be assumed that species are infected at varying prevalences, i.e. in some species nearly all individuals might be infected while in other species only a small proportion harbors *Wolbachia*. Thus, the parameter describing the prevalence among species has its own probability distribution. In each species sample the number of individuals tested positive is still binomially distributed, but the parameters vary: the prevalence differs from species to species and moreover, for each species the number of tested individuals can differ. The beta-binomial model provides an appropriate method within which the data can be analyzed as a whole. The binomially distributed random variables X_j correspond to the number of individuals tested positive for the single species. The different prevalences q_j are assumed to follow a beta distribution. This assumption is purposive because, depending on the parameters α and β, the shape of a beta distribution can vary widely (Fig. 5.2). Since almost nothing is known about the real prevalence distributions, it is expedient to leave many possible forms for the potential prevalence distributions. Then, to obtain a certain number k of infected individuals X from any species sample has a certain probability given the beta distributed parameter q, $P(X = k|q)$ which is then beta-binomially distributed.
We use the data from *Wolbachia* screenings to estimate the parameters in the beta-binomial model and to eventually obtain an estimate of the beta function describing the prevalence distribution. The beta distribution depends on two parameters α and β. It was shown that mean and variance of the beta-binomially distributed random variable X can be described in terms of α and β (equations 5.1 and 5.2):

$$\mu = \frac{\alpha}{\alpha+\beta} \qquad \text{and} \qquad s^2 = \frac{\alpha\beta}{n_j(\alpha+\beta)^2}\left(1+\frac{n_j-1}{\alpha+\beta+1}\right).$$

Mean and variance are typically estimated by moment estimators calculated

from available data. For equal n_j, one would use the standard first and second moment estimator to determine μ and s^2. In our case the sample sizes vary because for different species different numbers of individuals were tested. Therefore we use weighted moment estimators (Carlin and Louis, 2000)

$$\hat{\mu} = \frac{\sum_{j=1}^{N} X_j}{\sum_{j=1}^{N} n_j} \quad \text{and} \quad \widehat{s^2} = \frac{N \sum_{j=1}^{N} n_j \left(\frac{X_j}{n_j} - \hat{\mu}_j\right)^2}{(N-1) \sum_{j=1}^{N} n_j},$$

where N is the number of species tested. With these estimates, α and β can be calculated by solving the following system of equations

$$\hat{\mu} = \frac{\alpha}{\alpha + \beta} \quad \text{and} \quad \alpha + \beta = \frac{\widehat{s^2} - \hat{\mu}(1-\hat{\mu})}{\frac{N\hat{\mu}(1-\hat{\mu})}{\sum_{j=1}^{N} n_j} - \widehat{s^2}}$$

and the prevalence distribution is determined.

5.2.3 Estimation Procedure

The following estimation procedure is applied to available data from *Wolbachia* screenings. We obtain estimates of a beta distribution describing the prevalence distribution among species. Based on this beta distribution, estimates of the overall infection frequency of *Wolbachia* can be derived.

1. Determination of moment estimators

$$\mu = \frac{\sum_{j=1}^{N} X_j}{\sum_{j=1}^{N} n_j} \quad \text{and} \quad \widehat{s^2} = \frac{N \sum_{j=1}^{N} n_j \left(\frac{X_j}{n_j} - \hat{\mu}\right)^2}{(N-1) \sum_{j=1}^{N} n_j},$$

   where X_j is the number of infected individuals, n_j is the number of individuals tested of species j and N is the number of tested species.

2. Determination of α and β by the following equations:

$$\hat{\mu} = \frac{\alpha}{\alpha + \beta} \quad \text{and} \quad \alpha + \beta = \frac{\widehat{s^2} - \hat{\mu}(1-\hat{\mu})}{\frac{N\hat{\mu}(1-\hat{\mu})}{\sum_{j=1}^{N} n_j} - \widehat{s^2}}.$$

3. Determination of the incidence $\hat{x}$ by integrating the distribution of the prevalence which is a function of both estimated parameters α and β:

$$\hat{x} = \int_c^1 \frac{1}{B(\alpha, \beta)} q^{\alpha-1} (1-q)^{\beta-1} dq,$$

   where c defines a threshold value above which species are considered infected.

Data sets	α	β	$\mu = \hat{q}$	Incidence $\hat{x}$ in %		
				$c = 1\%$	$c = 0.1\%$	$c = 0.01\%$
(i)	0.32	0.41	44.7	85.1	92.9	96.6
(ii)	0.39	0.68	36.1	86.1	94.3	97.7
(iii)	0.12	0.36	25.3	51.7	61.6	**6**9.7
(iv)	0.18	0.52	26	62.6	**7**4.5	**8**2.6
(v)	0.11	0.36	23.3	47.2	56.7	64.5

Table 5.4: Parameter estimates α, β and μ for the different data sets are shown. μ is the estimate for the average prevalence of infection. Estimates for the incidence are given for three different values of c, which is the minimum infection density above which a species is considered infected. Numbers printed in bold indicate that estimates are consistent with data from one-individual samples (i.e. $\chi^2 < 3.84$).

By weighting the infection densities with the particular sample size, large samples have strong impact on the estimation procedure. Therefore we determined distributions $B_{(i)}$, $B_{(ii)}$ and $B_{(iii)}$ for the prevalence of *Wolbachia* based on three different data sets: (i) complete data, (ii) without the *C. pipiens* sample (thus $n_j < 1000$) and (iii) samples with sample size $n_j < 100$. Thereby we can check if and to which degree the potentially biased large samples influence the results. Also, we tested a data set excluding samples from those species that were known to be infected and analyzed to determine within species infection dynamics. In analogy to the three first data sets, also here we test for the influence of large samples. Data set $B_{(iv)}$ includes all samples from species that were not definitely known to be infected before and $B_{(v)}$ consists only of those with $n < 100$ (the *C. pipiens* example was excluded before because infection was known).

5.3 Results

5.3.1 Prevalence Distributions

All the five resulting beta functions show a 'most-or-few' infection pattern, as very high as well as very low intraspecies infection frequencies are more likely to occur than infection frequencies inbetween (Fig. 5.3). We accentuate that this is a first important result, because a beta distribution potentially can take various forms as illustrated by Figure 5.2. Since all five data sets produce estimates of the beta distribtion that conformly show such a U-shaped distribution, there is strong evidence that *Wolbachia* infections either occur at very high or very low prevalence within species.

5.3.2 Incidence Estimates

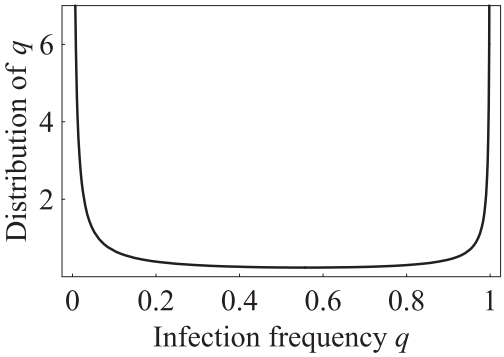

Figure 5.3: Estimated prevalence distribution based on the data set $B_{(iii)}$.

The estimate of the incidence, i.e. the total proportion of infected species, is obtained by integrating the obtained prevalence distributions. The actual estimate, however, depends on an arbitrarily chosen parameter c, which defines the threshold value below which species are considered uninfected. This is necessary because the beta distribution is a continuous function, not providing a criterion to distinguish between infected and uninfected species by itself. The arising problem is that the different beta functions as well as different values of the threshold value c produce very different estimates of the incidence ranging from 56 to 98% (Table 5.4). To compensate for these variations and to choose the best candidate representing *Wolbachia* infection dynamics, we apply two χ^2-tests to supposably unbiased subsets of the data. First, we test if the estimates are consistent with data from the one-individual samples. One-individual samples might represent independent data because species were predominantly randomly chosen, without prior knowledge of the infection status (e.g. Werren et al. (1995a)). Second, we use larger samples as representatives for the prevalence distribution. By applying another χ^2-statistic we test whether predictions based on our obtained beta distribution are consistent with collected data .

Testing the Incidence Estimates

Among the one-individual samples, 110 of 522 species were found to be infected. We use this as a reference value to test the above obtained estimates. By testing if the estimated beta distribution could represent the underlying density functions, it is incorporated that some of the negatively tested species might well be infected: Let q be the average prevalence, i.e. the average infection frequency within species and let x be the total proportion of infected species. Now assume that we pick randomly one individual of any species. With probability x, we have picked an infected species. The probability that we also picked an infected individual is now q. Thus, the probability of randomly obtaining an infected individual is qx. Analogously,

the probability to obtain an uninfected individual is $1 - qx$. Among these, however, a proportion $\frac{x(1-q)}{1-qx}$ represents the 'false negatives', species that are infected but were wrongly classified as uninfected because the representative picked did not harbor a *Wolbachia* infection. Our estimation procedure provides an estimate of the average prevalence and incidence depending on the threshold value c for each data set. With the data from the one-individual samples, each pair (q, x) can be tested for consistency with the observations by simply applying a χ^2-test. The following function describes this χ^2-value as a function of both parameters q and x

$$\chi^2(q,x) = \frac{(522qx - 110)^2}{522qx} + \frac{(522(1-qx) - 412)^2}{522(1-qx)}, \tag{5.3}$$

based on the observations from one-individual samples. Pairs (q, x), for which $\chi^2(q, x) < 3.84$ can be accepted (with an error probability of 5%).

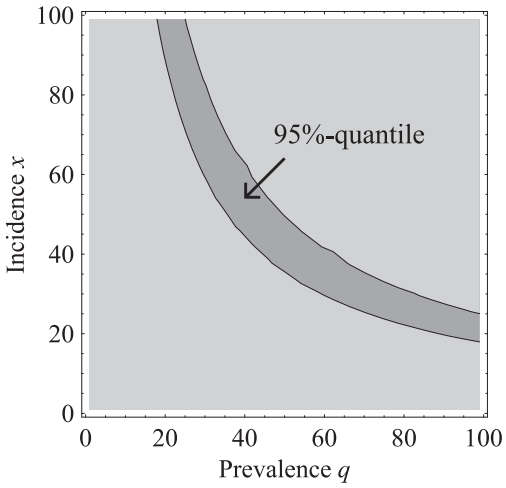

Figure 5.4: The 95%-quantil for pairs (q, x) is shown: Pairs laying within the dark gray area are consistent with the one-individual samples.

Figure 5.4 shows the 95%-quantile for pairs (q, x) that can be accepted as parameters describing the underlying distribution of *Wolbachia* infections based on data from one-individual samples. Table 5.4 shows several estimates of q and x for different values of c. Estimates printed in bold indicate pairs (q, x) laying inside the 95%-quantile, thus estimates that can be accepted as describing the underlying *Wolbachia* distribution. Table 5.4 further shows that the estimates obtained from data sets $B_{(i)}$ and $B_{(ii)}$ are inconsistent with observed data for chosen values of the parameter c. Both overestimate the incidence of *Wolbachia*, estimating up to almost 98% species to be infected. Both data sets contain the 13 ($B_{(i)}$) or 12 ($B_{(ii)}$) large samples ($n > 100$), of which only one species was found to be uninfected. Apparently, this results in a strong bias towards an overestimation of the overall infection frequency. Figure 5.5 shows the impact of the threshold value c on the incidence estimates. Of course, incidence estimates decrease with increasing c. Data sets $B_{(iii)}$ and $B_{(iv)}$ both provide incidence estimates consistent with one-individual samples for small $c \leq 10^{-4}$. In contrast, $B_{(i)}$ requires $c \geq 0.3$ to produce reliable incidence estimates. To consider a species

as infected only for a minimum prevalence of 30% is doubtlessly overstated and there are several examples of prevalences of less than 30% (Tagami and Miura, 2004, Jiggins et al., 2001b). Comparison of data sets depending on the maximum sample size shows that excluding large samples leads to smaller incidence estimates. This is true for data sets $B_{(i)}$-$B_{(iii)}$ comprising all samples of the particular sample sizes. Also data set $B_{(iv)}$ (all n) estimates the incidence to be higher than $B_{(v)}$ ($n < 100$), although both exclude samples from species that were known to be infected before. However, the remaining five large samples in $B_{(iv)}$ show relatively small prevalences. Therefore, the estimated distribution suggests that low infection densities are common, but likely to be overlooked which also can result in an overstated incidence estimate. Since large samples always have such a strong impact, we conclude that using only $n < 100$ samples gives the best estimates of the overall percentage of infected species.

Giving an actual number for the incidence of *Wolbachia* is difficult, because estimates depend on the composition of data sets and the parameter c. We decide to use the smallest obtained estimates as final incidence estimators. Besides values listed in Table 5.4 of which we would choose the 69.7% estimate, we obtained another estimate of 67.8% for c=0.4% and data set $B_{(iv)}$. Both are consistent with one-individual samples, while lower estimates for bigger values of c are inconsistent. Thus, both might represent a lower bound for incidence estimates emerging from the particular data sets. Finally, we conclude that the incidence of *Wolbachia* can be estimated to be around 67-70%.

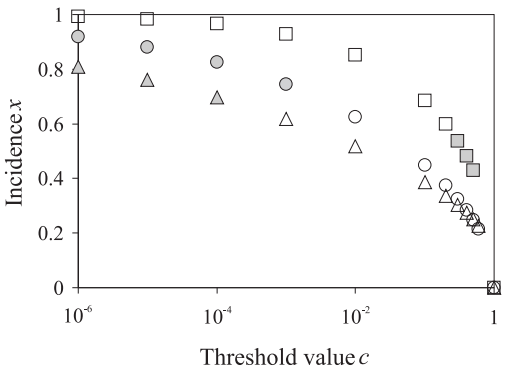

Figure 5.5: This graph shows incidence estimates of data sets $B_{(i)}$ (squares), $B_{(iii)}$ (triangles) and $B_{(iv)}$ (circles) depending on threshold value c. White symbols denote estimates being inconsistent with one-individual samples while gray symbols show consistent estimates.

Testing the Estimated Prevalence Distribution

We applied a further χ^2-test to check whether infection densities within species are appropriately represented by the estimated beta distributions. Therefore, we compared the observed infection densities in species of which at least 22 individuals were tested (by analyzing 22 individuals a prevalence of

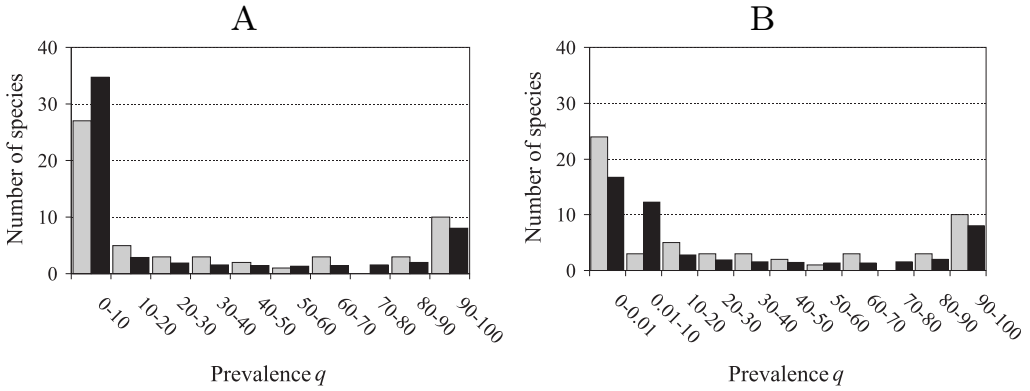

Figure 5.6: Numbers of species with infection densities in the particular intervals are shown. Gray bars describe the observations made in samples with sample size $n_i \geq 22$. The black bars indicate the number of species expectes based on $B_{(iii)}$. The value of the χ^2-statistic is 10.1 (< 14.07, error probability 5%), Thus we can accept this distribution as an underlying density function. Other graphic: $\chi^2 = 37.3 > 15.51$

10% is detected with a probability of 90%, thus these samples should be good representatives of the prevalence among species) with the expected number of species in certain prevalence ranges (Fig. 5.6) and applied a χ^2-test. This test confirmed that data set $B_{(iii)}$ provides reliable estimates (note that this is independent of the parameter c), but also the other beta distributions could have been accepted. This gives further support for the 'most-or-few' infection pattern to be valid. Figure 5.6 further points out another interesting fact. In graph A, we divided the the scale for the prevalence into sections of 10%. Then, uninfected as well as low prevalence infected species fall into the same interval and thus the proportion of infected versus uninfected species does not play a role. In this case, the χ^2-value implies that we can accept this function as an underlying distribution. If the interval from 0 to 10% percent is supplementary divided in a part in which species are considered uninfected and a part which represents infections of less than 10%, application of the χ^2-test suggests the rejection of this function. Graph B clearly shows that the difference between the observed and expected number of species in the particular intervals [0,0.01] [0.01,10] is high. This contributes strongly to the χ^2-statistic exceeding the critical value. However, we suppose that this is due to the fact that low prevalence infections have likely been overlooked, because for other intervals, expectation and observation match each other well.

To summarize, we state that *Wolbachia* infection dynamics obey a 'most-or-few' infection pattern, i.e. *Wolbachia* infections occur at either very high (>90%) or very low prevalence (<10%). Furthermore, it was shown that

previous incidence estimates of around 20% were underestimates and that it is rather the case that 67-70% of species are infected.

5.4 Discussion and Perspectives

In this chapter we presented a meta-analysis of published data on *Wolbachia* infections. This is the first such meta-analysis and it provides new insights into infection dynamics of *Wolbachia*. The main findings are that first, regarding infection frequencies within species, a 'most-or-few' infection pattern is likely to be valid, i.e. the prevalence is typically either very high (>90%) or very low (<10%). Second, incidence estimates of around 67%-70% indicate that previous incidence estimates of generally $\sim$ 20% were strong underestimations.

That the incidence of *Wolbachia* has likely been underestimated due to the non-detection of low prevalence infections has been mentioned in several studies (Werren et al., 1995a, Tagami and Miura, 2004, Jiggins et al., 2001b). This meta-analysis provides strong support for the proportion of species harboring *Wolbachia* being in fact significantly higher than 20%. Obviously, these estimates apply primarily to the available data (comprising 893 species after all) possibly not presenting a random choice of species. Further, giving a particular percentage is difficult because the estimator of the overall infection frequency depends on an arbitrarily chosen parameter (c). However, we obtained estimates that are consistent with the data from predominantly randomly sampled one-individual samples. Since also these consistent estimates differ, we chose the most 'careful' variant and estimate the total number of infected species to be around 67-70%. It should be noted that this result does not support the estimate of 76% infected species by Jeyaprakash and Hoy (2000), because our estimation is derived from studies that predominantly give infection rates for one-individual samples of around 20% whereas the Jeyaprakash and Hoy (2000) estimate gives a figure of 76% for predominantly one-individual samples. That study was excluded from this analysis because its one-individual sample estimates of infection are inconsistent with other studies, and their methods are likely more prone to false positives. In contrast, our result is consistent with other one-individual samples (Werren et al., 1995a, Werren and Windsor, 2000, West et al., 1998).

We further conclude that a 'most-or-few' infection pattern is likely to be valid for *Wolbachia*: either very few or most individuals of a species are infected (Fig. 5.3,5.6). It will be interesting to examine correlations between high or low prevalence infections and, for example, the type of manipulation induced by *Wolbachia*. It has been suggested that MK-*Wolbachia* typically

occur at low prevalence but CI-*Wolbachia* at high prevalence within populations (Hurst et al., 2000). So far, however, most studies on infection frequencies were restriced to detecting *Wolbachia* infections without studying effects on host populations. It is moreover possible that the structure of populations, i.e. several populations that are connected by migrating individuals, is responsible for high or low prevalence infections, respectively. CI-*Wolbachia* usually spread very easily through single host populations, but in parapatric populations equilibria of infected and uninfected can be established (Flor et al., 2007). In such cases, also CI-*Wolbachia* can occur at low to intermediate frequencies.

Some limitations of the meta-analysis must be recognized. Firstly, our statistical approach draws attention to the fact that the predicted percentage of infected species depends crucially on the minimum cut-off to categorize a species as infected (c). If we accept 1 of 10,000 individuals with an infection as defining an infected species, we will get a much different estimate than if we use 1 of 1000 as a cut-off. Secondly, data were collected from different laboratories and often using different *Wolbachia* specific primers for detection. This is a common issue with meta-analyses. It is encouraging that most larger broad taxon screening studies (e.g. > 50 species tested and not limited to a single host taxon) give one-individual infection rates within similar ranges of 15-30%. Thirdly, analyzed species might not represent a random choice. However, the statistical methods shown here also can be applied as data sets improve and more consistent methods across studies are employed. It is important to get better estimates of the distribution of infection frequencies within species. Thereby it will be important that more individuals per species are assayed for randomly chosen species, since we have shown that data from currently existing large samples bias the outcomes of statistical analyses towards a higher infection frequency of *Wolbachia*. However, caution should be exercised, as there will be a tendency to over-sample common species by this method, as large samples from common species are more easily collected. With sufficient data it will also be possible to compare the *Wolbachia* infection patterns among different arthropod taxa or across geographical regions. Furthermore, the statistical method used here can be applied to estimate infection dynamics of other infectious agents.

Chapter 6

Summary and Perspectives

The objective of this work was to continue investigations on *Wolbachia*'s role in arthropod evolution. That *Wolbachia* have the potential to influence host speciation has been shown in previous experimental (Breeuwer and Werren, 1990) and theoretical (Telschow et al., 2005a, Flor et al., 2007) studies. The theoretical studies are based on the Dobzhansky-Muller model but assume that populations become reproductively isolated due to the aquisition of different *Wolbachia* infections. The classical Dobzhansky-Muller model, however, considers reproductive isolation as a result of genetic divergence. Within this framework, it should therefore be the rule rather than the exception that allopatric populations also diverge genetically while, at the same time, acquiring *Wolbachia* infections. When studying *Wolbachia*'s role in speciation, it should thus be considered that *Wolbachia*-induced CI can occur simultaneously with nuclear incompatibilities and that both contribute to postzygotic isolation. These possible and probable interactions have not been examined before, and this work expands theoretical investigations to more generalized scenarios by taking into account the interactions of *Wolbachia*-induced CI with nucleus-based genetic incompatibilities.
Most studies on *Wolbachia*'s role in speciation have predominantly dealt with rather specific scenarios and most often suggested a strong impact for bidirectional CI only. Bidirectional CI acts as a strong postzygotic isolation mechanism and can forcefully promote speciation processes. Naturally occurring bidirectional CI has been reported in some insect species, but it is still unclear how frequent it occurs since it requires at least two subpopulations of one species to acquire different CI-inducing *Wolbachia* infections. In contrast, it appears more likely that unidirectional CI is expressed between subpopulations of one species because this requires only one population to become infected with CI-inducing *Wolbachia*. Therefore, in order to establish *Wolbachia* as a general factor in evolutionary processes, it is important to

show that also unidirectional CI can drive speciation.
In chapters 3 and 4 we focused on the question of how CI interacts with nuclear incompatibilities and, in particular, under which conditions unidirectional CI can have an impact on speciation processes. An important characteristic of our model is its diploid genetic structure. Since the majority of species is diploid, our model is better applicable to most organisms than the often used simplified models with haploid genetics. Furthermore, diploidy is necessary to include dominance effects of incompatible alleles and, in particular, to implement recessive nuclear incompatibilities. In this way, we can formalize Muller's dominance theory to model sex-specific incompatibilities that obey Haldane's rule.
In chapter 3, we investigated the interactions of CI and typical Dobzhansky-Muller autosomal-autosomal incompatibilities. By analyzing NI alone, we found that reproductive isolation due to recessive incompatibilities is more easily lost than isolation caused by dominant incompatibilities. Regarding the interactions of CI and NI, our results show that bidirectional CI can strongly enhance genetic divergence under a broad range of conditions. As a result, even very weak recessive genetic incompatibilities can be maintained as isolating mechanisms. Remarkably, this effect is still strong if we allow asymmetries in the expression of bidirectional CI, which is generally expected to result in less robustness of CI as an isolating mechanism. Since recessive incompatibilities are supposed to occur much more frequently than dominant incompatibilities, the effect of *Wolbachia* in interaction with such weak nuclear incompatibilities is particularly important for the maintenance of genetic divergence. Moreover, we could show that unidirectional CI and genetic incompatibilities can stabilize each other when interacting. This generally happens under more restricted conditions, but for certain scenarios significant synergistic effects could be observed. As a result, both isolating mechanisms are significantly strengthened by co-occurring with the other.
In chapter 4 we examined a more specific scenario where nuclear incompatibilities act sex-specifically (Haldane's rule) and interact with unidirectional CI. In scenarios with heterogametic females (ZW sex determination, e.g. Lepidopterans), we found a strong increase in the stability of postzygotic isolation. Since Haldane-type NI always affects the heterogametic sex, the lethality of infected hybrid females acts as a barrier against the spread of maternally inherited *Wolbachia*. This results in CI being maintained as an isolating mechanism under a wider range of conditions, which can in turn stabilize nuclear incompatibilities. Conversely, such effects did not occur in taxa with XY sex chromosomes such as *Drosophila*, since lethality of infected hybrid males does not impede transmission and spread of *Wolbachia*.

From chapters 3 and 4 we can draw three main conclusions:

1. Recessive nuclear incompatibilities can show very different dynamics compared to dominant incompatibilities. Diploid modeling is therefore essential because recessive incompatibilities or sex-specific incompatibilities obeying Muller's dominance theory cannot be implemented in a simplified haploid model.

2. Bidirectional CI enhances genetic divergence. This is particularly important for the persistence of the most frequently occurring weak recessive nuclear incompatibilities.

3. Unidirectional CI and genetic incompatibilities can promote speciation processes more forcefully when interacting. In particular, unidirectional CI might have more impact on speciation processes in taxa with heterogametic females such as Lepidoptera.

From these results we can also derive suggestions for future theoretical models on *Wolbachia*'s role in speciation. One has to consider that *Wolbachia*-induced alterations of host reproduction interact with the incompatibilities that are caused by genetic differentiation. It will be interesting to expand our models to more than two incompatible loci, especially since it is not clear yet how many loci usually are involved in incompatibilities. Also, the potential of CI or NI as an initiating isolating mechanism can be investigated. Whether CI between subpopulations provides the possibility for subsequent genetic divergence could be examined in a model where subpopulations aquire *Wolbachia* infections, become connected by migration, and mutant alleles appear while migration takes place. Then one could analyze the influence of *Wolbachia* infections on subsequent accumulation of incompatible loci. In analogy, one could examine whether genetic incompatibilities in parapatric populations enhance or repress the establishment of *Wolbachia* infection polymorphisms. A further possible extension of our model is the integration of more populations. Once more studies have shed light on naturally occurring interactions of CI and NI within structured populations, these could serve for better, more realistic modeling approaches. Moreover, species-specific scenarios could be implemented based on observations on population structures and incompatibility types. Finally, we stress the importance of diploid modeling, especially when recessive effects are to be considered. This should most often be the case because most incompatible alleles are supposed to act recessively and, moreover, Muller's dominance theory requires recessivity of alleles to explain Haldane's rule. However, there are also many arthropod species with haplo-diploid genetics, e.g. Hymenoptera females are diploid

while males are haploid. Our diploid models do not match the genetics of such species. As we have seen in chapter 4 the sex determination system can crucially affect model dynamics, and it will be interesting to examine how dynamics change if other, different genetic architectures are considered.
Besides the potential impact of *Wolbachia*-induced CI on speciation, *Wolbachia*'s actual role in speciation processes will depend upon their abundance and the frequency of CI-*Wolbachia* in particular. In chapter 5 we have shown that current estimates of 20% infected species were underestimations and that it is more likely that about two-thirds of species are infected. We remark that our analyzes probably does not provide final estimates of *Wolbachia* infection frequencies because the available data might not represent an unbiased subset of arthropod species. However, we addressed some of the problems that arise from these sampling methods and also suggested methods for the collection of more reliable data. By applying our estimation procedure to such data sets, estimates of the incidence of *Wolbachia* would improve. Once sufficient data are available, the statistical method presented could also be applied to compare infection patterns in different taxonomical groups or geographical regions.
It will also be interesting to elaborate correlations between infection densities within species and the type of manipulation that is induced by *Wolbachia*. It is of central interest for *Wolbachia*'s role in arthropod speciation how many species are infected with CI-*Wolbachia*. CI is generally assumed to be the most common of the alterations *Wolbachia* are capable to induce. However, studies on the actual frequencies of different *Wolbachia*-types among infected species have not been conducted yet. Still, with *Wolbachia* infecting about two-thirds of species, expression of CI within arthropod species is certainly a common process and *Wolbachia* thus an influential and important factor in arthropod speciation processes.
There is another bacterial group that can cause similar alterations in their host species. Bacteria *Cardinia* were found to cause cytoplasmic incompatibility in a wasp species (Hunter et al., 2003). The interaction of nuclear and cytoplasmic incompatibilities is thus not restricted to species infected by *Wolbachia* but can occur in the presence of other bacteria as well. Future studies might find further cytoplasmic endosymbionts that can interfere with their hosts' reproduction and will describe these symbionts' influence on arthropod speciation processes more precisely.
Within the last 20 years, research on speciation has made great progress. Current molecular techniques allow profound analyses of genetics of organisms and the identification of genes that cause speciation processes. At the same time, it is possible to detect and analyze cytoplasmic elements and their potential impact on host species and particularly on host evolution.

However, evolutionary processes are equally intriguing as complicated, and many open questions remain. This thesis might motivate the simultaneous consideration of both genetic and cytoplasmic components in future studies which will hopefully help to improve our understanding of species richness.

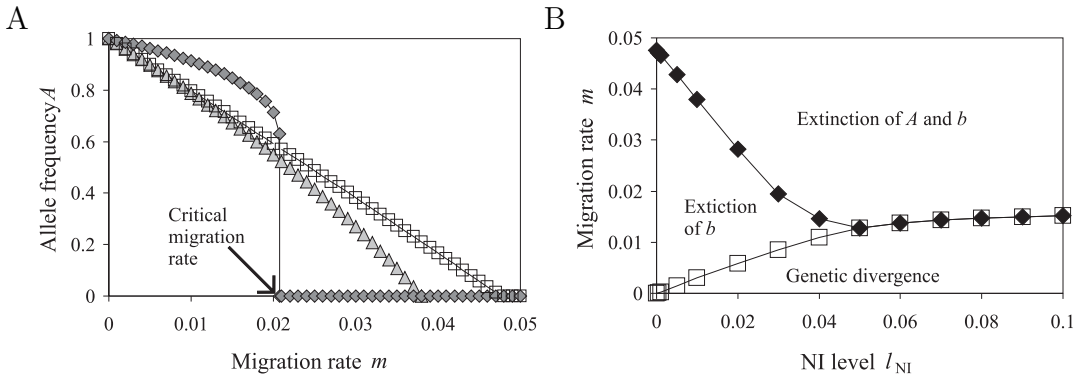

Figure A.1: Threshold values for the maintenance of genetic divergence. Graph A shows equilibrium frequencies of A as functions of the migration rate for dominant NI with NI levels of $l_{\mathrm{NI}} = 0$ (boxes), $l_{\mathrm{NI}} = 0.01$ (triangles) and $l_{\mathrm{NI}} = 0.5$(diamonds): Graph B shows migration rates below which alleles A (diamonds) and b (boxes) become extinct. Parameters are $h = 1$ and $s = 0.1$.

A Weak NI

The analysis of dynamics in the classical Dobzhansky-Muller model has shown that there are certain parameter values for which critical migration rates cannot be determined, at least critical migration rates do not fulfill the criterion of describing a significant change from coexistence of all four alleles to the state where two alleles have gone to extinction. This can happen when NI is weak and/or local viability selection is comparably strong. As shown by Figure A.1, equilibrium frequencies of A decrease continuously with increasing migration rate if there is no NI. If NI is weak ($l_{\mathrm{NI}} = 0.1$), equilibrium frequencies of A decrease with increasing migration as well, however, NI involve A, whereas a is not affected and A becomes extinct for lower migration rates than without being affected by NI (Fig. A.1A). A significant change of the frequencies of allele A p_A is seen at a certain value of the migration rate for stronger NI levels. With increasing migration rate, p_A decreases continuously until the critical value is reached. At this point, A is still present on the island at a frequency of generally 50% or more. If migration exceeds this value, A becomes completely replaced by a. Allele b benefits from NI, because in contrast to the competing allele B, it is only passively affected (through A). Thus, when nuclear incompatibilities build up a barrier against B to spread, also b and B can coexist up to a certain value of the migration rate. This value is, for very low NI levels, smaller than the threshold value for the coexistence of A and a since A is favored by local selection while b is not. Therefore, within this parameter range, A remains in the population up to higher migration rates than b (Fig. A.1B). Above a certain NI level, however, there is only one critical value of the migration rate below which

all four alleles coexist and above which residents $AAbb$ become extinct. This threshold value of the migration rate is what we referred to as the critical migration rate.

For recessive incompatibilities, Figure A.2 shows that although such a significant frequency change occurs, allele A does not need to have completely vanished. For weak NI, frequency of A jumps to a significantly lower, but positive value and converges to zero with increasing migration rate. This is also true for codominant and dominant NI, but only if NI level is very low. For recessive NI, this holds for every NI level but frequencies of A for migration rates exceeding the critical value are significantly lower for higher NI levels. We remark that allele b becomes completety extinct as soon as the critical migration rate is exceeded. Therefore, and because A-frequencies are generally low in this parameter range, the critical value is again refered to as the critical migration rate.

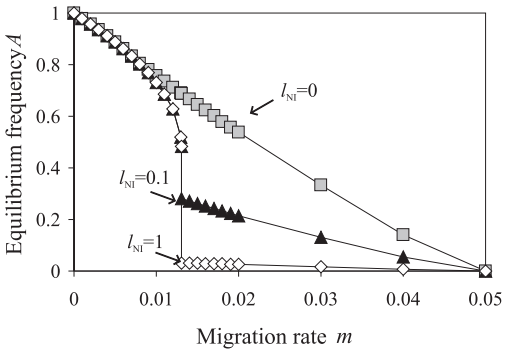

Figure A.2: Frequencies of A for recessive NI. Shown are equilibrium frequencies of A as functions of the migration rate for recessive NI for $l_{\mathrm{NI}} = 0$ (boxes), $l_{\mathrm{NI}} = 0.1$ (triangles) and $l_{\mathrm{NI}} = 1$(diamonds). Other parameters are $h = 0$ and $s = 0.1$.

B Symmetric vs. Asymmetric Incompatibilities

In many theoretical studies nuclear incompatibilities are modelled in many different ways. We assumed asymmetry of incompatibilities, i.e. A and B are incompatible while a and b are compatible. Nuclear incompatibilities are supposed to be asymmetric in the beginning of diverging process but accumulation of further incompatible loci can result in symmetric incompatibilities. For completeness, few results from a model where hybrid lethality might also be caused by a and b are presented. Let's assume that l_{AB} (as throughout chapter 4) is the proportion of inviable offspring $AABB$ and further l_{ab} the proportion of inviable offspring $aabb$ (Table B). For simplicity, only one dominance level h is defined. For $h = 0$, deleterious effects are recessive and only F_2 hybrids $aabb$ and $AABB$ are inviable. For dominant NI ($h = 1$), reproductive isolation is perfect and no hybrids are produced. Thus, both genotype groups could coexist up to a critical migration rate of $m_c = 0.17$ without local selection acting on alleles (see 2.3.2). Otherwise, critical migration rates for nuclear divergence range from critical migration rates for completely asymmetric NI presented above and those for the case of reproductive isolation. Again, critical migration rates increase with increasing strength of incompatibility levels and strength of local selection.

However, there is one basic difference in dynamics. If only a small proportion of hybrids $aabb$ is inviable, genetic divergence is maintained even without local selection acting. This is because all alleles are affected by NI. Let us compare dynamics of allele a in both scenarios without local selection. If NI are completely asymmetric ($l_{ab} = 0$), there is no barrier acting to prevent a from spreading on the island. If A is not locally selected, it has no fitness avantage over a which benefits from continuous genetic influx. Thus, a replaces A. If a is affected from incompatibilites, A can coexist with a as long as the genetic influx from the mainland is sufficiently weak. Thus, genetic divergence is possible below a certain critical migration rate (Fig. B.1A).

	aa	aA	AA
bb	$1 - l_{ab}$	$1 - hl_{ab}$	1
bB	$1 - hl_{ab}$	$1 - hl_{abAB}$	$1 - hl_{AB}$
BB	1	$1 - hl_{AB}$	$1 - l_{AB}$

Table B.1: Possible modeling of symmetric NI. For simplicity, it is assumed that the dominance level h is the same for all alleles.

For recessive NI, incompatibility of a and b in addition to that between A and B can strongly influence stability of postzygotic isolation. Although critical migration rates are robust against switches from complete asymmetric

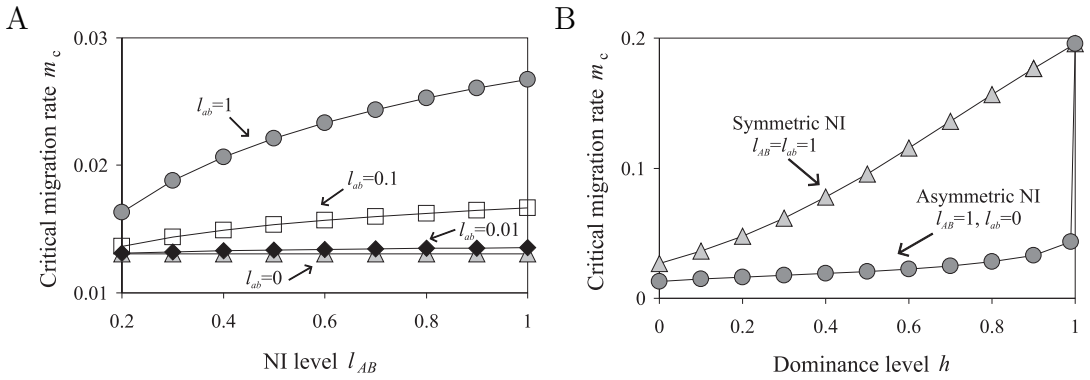

Figure B.1: Asymmetric versus symmetric NI. Graph A shows critical migration rates for asymmetric to symmetric incompatibilities for recessive NI. Parameters are $l_{AB} = 1$, $h = 0$ and $s = 0.1$. Graph B shows critical migration rates for completely asymmetric and symmetric NI as functions of the dominance level. Other parameters are $s = 0.1$ and $l_{abAB} = l_{AB}$

($l_{ab} = 0$) to almost asymmetric ($l_{ab} = 0.01$) NI (Fig. B.1A), a large deviation is seen if a and b are completey incompatible ($l_{ab} = 1$). Critical migration rates change from $m_c = 0.0131$ for the total asymmetric case ($l_{ab} = 0$) to $m_c = 0.027$ for the symmetric case ($l_{ab} = 1$). For codominant or dominant NI discrepancy becomes even larger. With increasing dominance, critical migration rates for symmetric NI increase more steeply than for asymmetric NI (Fig. B.1B). Compared to the model where only A and B cause hybrid lethality and hybrids from interpopulation matings can be perfectly fit (i.e. $aAbB$), in the model with symmetric NI all recombined genotypes cause inviabilty. Thus, reproductive isolation is much stronger.

In both models, stability of postzygotic isolation increases with increasing strength of local selection. Difference between critical migration rates for symmetric and asymmetric NI decreases because the stronger local selection, the weaker the impact of the NI level. However, for reasonable values of selection coefficient ($0.01 < s_A < 0.1$), differences are still significant.

C Interactions of CI and Dobzhansky-type NI in Two-Way Migration Models

The model

The Dobzhansky-Muller model and the version including *Wolbachia* infections (section 3.2.1) is extended in order to incorporate bidirectional migration. Therefore it is sufficient to alter and extend equations 3.1 for the migration step and 3.2 and 3.3 for local viability selection. Besides $p_{\vec{i}}$ describing frequencies in the island population, let $q_{\vec{i}}$ denote the corresponding frequencies in the mainland population. Then frequencies after the migration step can be formalized by

$$\begin{aligned} p_{\vec{i}}^{+} &= (1-m_1)p_{\vec{i}} + m_1 q_{\vec{i}} \ . \\ q_{\vec{i}}^{+} &= (1-m_2)q_{\vec{i}} + m_2 p_{\vec{i}} \end{aligned} \tag{6.1}$$

Note that 6.1 simplifies to 3.1 if $m_2 = 0$. In this case, it is $q_{\vec{i}} = 1$ if $\vec{i} = (0,1,1)$ and $q_{\vec{i}} = 0$ otherwise.
Equivalently to the mainland-island model, allele A is positively selected in the island population and corresponding equations equal 3.2 and 3.3. In the two-way migration model, B is positively selected in the other population. Factor S_B is defined in analogy to S_A by

$$S_B(i,s) = \begin{cases} 1+s & if\ i=1 \\ 1+\frac{1}{2}s & if\ i=2 \\ 1 & else \end{cases} \ .$$

Frequencies after selection are described by

$$q_{\vec{i}}^{*} = \frac{q_{\vec{i}}^{+} S_B(i_2, s_B)}{W^*}$$

with $W^* = \sum_{\vec{i}} p_{\vec{i}}^{+} S_B(i_2, s_B)$. Since reproduction happens in each population without hybrid incompatibilities being affected by the environment, frequencies of subsequent generations are obtained by equations 3.5 and 3.6 as in the one-way migration model.

Results

Without *Wolbachia*

In analogy to the mainland-island model, the maintenance of genetic divergence is not possible without local selection unless reproductive isolation is

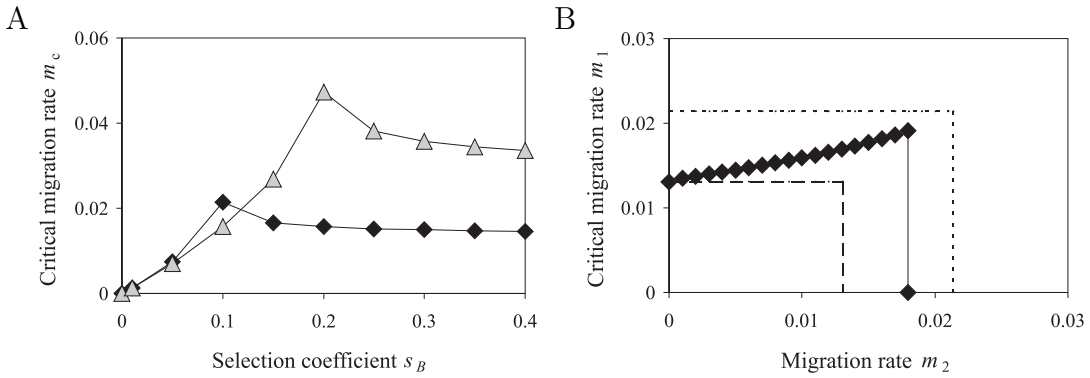

Figure C.1: Asymmetric selection or migration. Graph A shows critical migration rates as functions of the selection coefficient s_B, while s_A is held constant for $s_A = 0.1$ (diamonds) and $s_A = 0.2$ (triangles). Other parameters are $l_{\mathrm{NI}} = 1$ and $h = 0$. Graph B shows critical migration rates m_1 as function of a constant migration rate m_2. The dotted lines describe the value of the critical migration rate for the completely asymmetric ($m_2 = 0$) and symmetric ($m_1 = m_2$) case. Further it is $l_{NI} = 1$, $h = 0$ and $s_A = s_B = 0.1$.

perfect. Both alleles A and B become extinct during secondary contact because they are involved in incompatibilities whereas a and b are not and are therefore advantageous over A and B. Thus, there is a return to the ancestral genotype *aabb*. Symmetric local selection on A and B ($s_A = s_B$) allows the coexistence of all four alleles. If selection is very strong compared to incompatibilities both acting on A and B it can happen that individuals benefit more from carrying incompatible alleles A and B than compatible alleles a and b. Then, both ancestral alleles become extinct. For biological relevant parameters and most of the parameter range, however, the dynamics are equivalent to those in the mainland-island models: a hybrid zone is maintained up to a certain migration rate and above this migration rate one genotype becomes extinct. The value of selection coefficients further affects the stability equivalently to mainland-island model: the stronger local selection, the higher the critical migration rates. Asymmetric local selection ($s_A \neq s_B$) tends to destabilize the system (Fig. C.1A). Critical migration rates are at maximum if local selection is equally strong in both populations. Small changes, especially reduction of one of the selection coefficients can decrease the critical migration rates dramatically. Obviously, the greater selection coefficient favors the corresponding genotype. So, if migration exceeds the critical value, individuals *AAbb* spread if it is $s_A > s_B$ but become extinct if $s_A < s_B$. In two-way migration models also migration can be asymmetric. Generally, critical migration rates are higher for symmetric migration, such that asymmetries between the migration rates tend to reduce stability like asymmetries in the selection coefficients. However, this reduction is weak. Critical migration rates lay between the critical migration rates in

the one-way migration model which is the perfect asymmetric case and critical migration rates for the symmetric two-way migration model (Fig. C.1B).

Effect of CI

Two-way migration models with CI only have been analyzed (Telschow et al., 2005b, Flor et al., 2007) (see 2.3.2). We start with symmetric bidirectional CI. Critical migration rates for coexistence of different *Wolbachia* strains can be determined analytically and increase linearly from 0 if $l_{\mathrm{CI}} = 0$ up to $m_c = 0.196$ for $l_{\mathrm{CI}} = 1$. This matches results from mainland-island models (allthough critical migration rates are slightly higher in two-way migration models). So do model outcomes on interaction of CI and NI equal those from mainland-island models. *Wolbachia* significantly increase critical migration rates for nuclear divergence. For a selection coefficient of $s_A = s_B = 0.1$, these are always elevated up to $m_c = 0.216$ (compared to $m_c = 0.196$ for one-way migration models) for perfect CI ($l_{\mathrm{CI}} = 1$). Thus, stabilizing effect can be even stronger in two-way migration models because coexistence of *Wolbachia* strains is generally possible for higher migration rates than in mainland-island models.

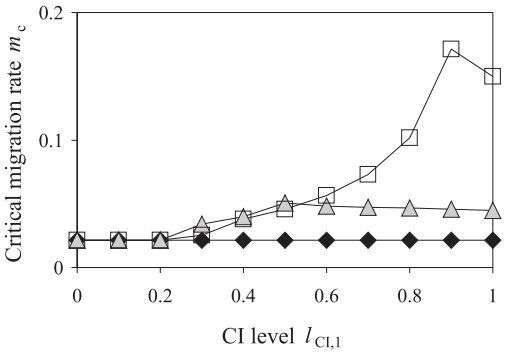

Figure C.2: Asymmetric CI reinforces NI. Shown are critical migration rates for nuclear divergence as functions of one CI level while the other CI level is held constant $l_{\mathrm{CI},0} = 0.1$(diamonds), $l_{\mathrm{CI},0} = 0.5$ (triangles) and $l_{\mathrm{CI},0} = 0.9$ (boxes). Other parameters are $s = 0.1$, $h = 0$ and $l_{\mathrm{NI}} = 1$.

Asymmetries in CI levels induced by different *Wolbachia* strains W_0 and W_1 generally results in reduced stability of infection polymorphism. This holds for one-way as well as for two-way migration models. In two-way migration models, critical migration rates are at maximum for symmetric CI ($l_{\mathrm{CI},0} = l_{\mathrm{CI},1} = l_{\mathrm{CI}}$) and decrease with increasing asymmetry (see equation 2.7 in 2.3.2). Asymmetric CI is capable of stabilizing NI, but since critical migration rates for asymmetric CI are generally lower than those for symmetric CI, this stabilizing effect is weaker. If one *Wolbachia* strain induces very weak CI ($l_{\mathrm{CI}} = 0.1$), *Wolbachia* infection does not provoke stability increase of nuclear divergence (Fig. C.2). However, if both strains induce intermediate to strong CI, there is a significant reinforcement of nuclear divergence.

Finally, unidirectional CI in a two-way migration scenario is considered. Critical migration rates for infection polymorphism can be approximated by the

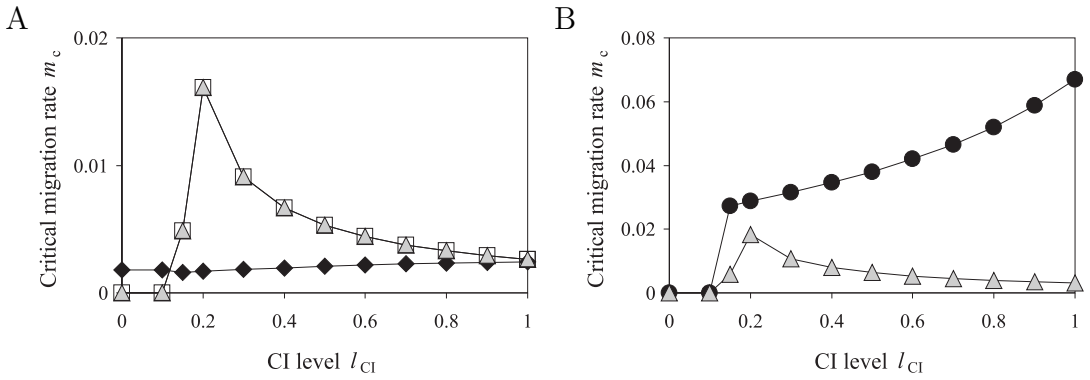

Figure C.3: Unidirectional CI interacting with NI in a two-way migration model. Graph A shows critical migration rates as functions of the CI level for nuclear divergence (diamonds) and infection polymorphism alone (boxes) and interacting with NI (triangles). Other parameters are for $s = 0.01$, $h = 0$ and $l_{\mathrm{NI}} = 1$. Graph B shows stability increase of infection polymorphism for $h = 0$ (triangles) and $h = 0.99$ (circles) as functions of the CI level. Other parameters are $l_{\mathrm{NI}} = 1$ and $s = 0.1$.

minimum of analytically derived critical migration rates in the one-way migration models (see 2.3.2). Critical migration rates in the two-way migration model are highest for CI levels around 20%. If CI level de- or increases, critical migration rates decrease. Thus, critical migration rates are approximated by those from mainland island models with infected island for a smaller range of CI levels from 0-20% and by those from mainland island models with infected mainland for other values (Flor et al., 2007). In the one-way migration models noticable effects in the interactions were mainly observed for CI levels stronger than 20%. This applies likewise in the two-way migration model. Therefore, interactions of NI and CI are similar to dynamics in the particular mainland-island model with an infected mainland and we summarize results here only briefly. Unidirectional CI can cause an increase in stability of genetic divergence if local selection is weak (Fig. C.3A). The same figure illustrates that recessive NI has no effect on stability of infection polymorphism. This is due to both, weak local selection and recessivity of NI. For stronger local selection and high dominance levels, i.e. strong NI, infection polymorphism generally collapses at lower migration rates than genetic divergence. (Fig. C.3B).

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Deutschsprachige Zusammenfassung

Einleitung

Diese Arbeit beschäftigt sich mit dem Einfluss intrazellulärer Bakterien der Gattung *Wolbachia* auf Artbildungsprozesse ihrer Wirte. *Wolbachien* sind weit verbreitet und infizieren zahlreiche Arthropoden, insbesondere Insekten (Hilgenboecker et al., 2008, Werren et al., 1995a). *Wolbachien* befinden sich typischerweise im Zytoplasma der Reproduktionsorgane ihrer Wirte. Sie können nur über die zytoplasmaenthaltenden Eizellen, aber nicht über Spermien von einer auf die nächste Wirtsgeneration übertragen werden. Demnach erfolgt die Vererbung der Endosymbionten durch Mütter, aber nicht durch Väter. Für das Bakterium stellen Männer also evolutionäre Sackgassen dar, da sie nicht mehr direkt auf die nächste Wirtsgeneration übertragen werden können. Infizierte Frauen hingegen sichern das generationsübergreifende Fortbestehen der Bakterien, weshalb ein hoher Anteil von infizierten Frauen in der Wirtspopulation von Vorteil für das Bakterium ist. So sind *Wolbachien* auch in der Lage die Reproduktion ihrer Wirte so zu manipulieren (siehe Werren (1997) für einen Überblick), dass das Geschlechterverhältnis zugunsten infizierter Frauen verschoben wird. Vier solcher Manipulationsstrategien sind bekannt, darunter die Induktion einer zytoplasmatischen Paarungsinkompatibilität (CI). Im allgemeinen unterscheidet man zwischen unidirektionaler und bidirektionaler CI. Unidirektionale CI tritt auf, wenn sich infizierte Männchen mit nichtinfizierten Weibchen paaren und resultiert in einer reduzierten Anzahl gemeinsamer Nachkommen. Alle anderen Paarungen, zwischen zwei infizierten, zwei nichtinfizierten oder zwischen infizierten Weibchen und nichtinfizierten Männchen sind kompatibel und produzieren die übliche Anzahl gesunder Nachkommen. Auf diese Weise verringern *Wolbachien* den Fortpflanzungserfolg nichtinfizierter Weibchen. Diese können sich nur mit nichtinfizierten Männchen fortpflanzen, den infizierten Weibchen gelingt dieses sowohl mit infizierten als auch mit nichtinfizierten Paarungspartnern. Bidirektionale CI tritt auf, wenn Paarungspartner mit verschiedenen

Wolbachia-Stämmen infiziert sind und äußert sich ebenfalls in einer meist stark reduzierten Anzahl gemeinsamer Nachkommen. Auch hier verhindern die einzelnen *Wolbachia*-Stämme, dass sich anders infizierte potentielle Wirte erfolgreich fortpflanzen und so das Fortbestehen konkurrierender *Wolbachia*-Stämme sichern.
Zytoplasmatische Inkompatibilität hat als mögliche Ursache für Artbildungsprozesse von Arthropoden Beachtung gefunden (Laven, 1959, Werren, 1998). In der klassischen Artbildungstheorie geht man davon aus, dass genetische Unterschiede zwischen Paarungspartnern zu partieller oder vollkommener Lethalität oder Sterilität des Nachwuchses führen können (Dobzhansky, 1940, Muller, 1942). Von *Wolbachien* induzierte zytoplasmatische Inkompatibilität kann also die gleichen Auswirkungen auf ihre Wirte haben wie genetische Unterschiede, die allgemein als Initiatoren von Artbildungsprozessen anerkannt werden. Daher liegt es nahe, einen möglichen Einfluss der Bakterien auf Artbildungsprozesse ihrer Wirte zu untersuchen.
Einige experimentelle (Breeuwer and Werren, 1990) und theoretische (Telschow et al., 2002; 2005a) Arbeiten haben gezeigt, dass bidirektionale CI einen starken Einfluss auf Artbildungsprozesse haben kann. Ob *Wolbachien* allerdings eine allgemeine Bedeutung in der Evolution von Arthropoden spielen können ist noch strittig (Hurst and Schilthuizen, 1998, Werren, 1998). Zum einen wird argumentiert, dass bidirektionale CI nur selten auf natürliche Weise vorkommt, da es die Infektion zweier Gruppen einer Wirtsart mit verschiedenen *Wolbachia*-Stämmen erfordert. Unidirektionale CI hingegen sollte vermehrt auftreten, da die Infektion einer Gruppe ausreichend ist. Damit *Wolbachia* als allgemeine Faktoren in Artbildungsprozessen etabliert werden können, sollte also auch ein Einfluss von unidirektionaler CI nachgewiesen werden. Theoretische Untersuchungen zur Rolle der *Wolbachien* in der Artbildung basieren auf der Struktur des klassischen Dobzhansky-Muller Modells. Das ursprüngliche Dobzhansky-Muller Modell beschreibt die Evolution nuklearer Inkompatibilität (NI): Man betrachte eine Population die durch einen Genotyp *aabb*, also zwei Allele auf zwei Loki, beschrieben wird. Diese Population unterteilt sich in zwei geographisch isolierte Subpopulationen. In jeder Untergruppe tritt nun ein Mutantenallele A bzw. B auf, breitet sich aus und ersetzt schließlich das ursprüngliche Allel a bzw. b. Nach diesem Prozess haben die Subpopulationen unterschiedliche Genotypen $AAbb$ bzw. $aaBB$. Jetzt werden beide Subpopulation wieder miteinander verbunden. Typischerweise wird dabei angenommen, dass Individuen zwischen den Populationen migrieren können. Dabei kann es zu Hybridpaarungen zwischen $AAbb$- und $aaBB$-Individuen kommen. Da die neuen Allele nicht gemeinsam in einem Individuum aufgetreten sind, ist es möglich dass sie Inkompatibilitäten in den Hybriden $AaBb$ verursachen. Diese äußern sich meist in einer

verminderten Fertilität oder Überlebenswahrscheinlichkeit. Dieses klassische Modell beschreibt die Evolution von Inkompatibilitäten zwischen zwei autosomalen Loki. Es kommt außerdem häufig vor, dass einer dieser Loki nicht auf einem autosomalen, sondern dem Geschlechtschromosom X liegt. Nun können Männer und Frauen unterschiedlich von Inkompatibilitäten betroffen sein. Ersetzen wir den B-Lokus im klassischen Modell durch einen X-Lokus und nehmen an, dass Inkompatibilitäten nun zwischen den neu evoluierten Allelen A und X auftreten. Homogametische Hybride sind nach wie vor durch einen Genotyp $AaXx$ charakterisiert. Die heterogametischen, die nur ein X Chromosom tragen, werden nun durch AaX (oder (Aax)) beschrieben. Nehmen wir weiterhin an, dass der schädlich Effekt von X rezessiv auftritt, so sind nur die heterogametischen Hybride AaX, nicht aber die homogametischen $AaXx$ von Hybridinkompatibilitäten betroffen. Dieses Phänomen wurde häufig beobachtet und wird gemeinhin als Haldansche Regel bezeichnet (Haldane, 1922, Laurie, 1997, Orr, 1997).
In Analogie zu diesen genetischen Divergenzprozessen gehen Studien zu *Wolbachia* davon aus, dass zumindest eine Subpopulationen während der Trennung mit *Wolbachia* infiziert wird (Telschow et al., 2002, Flor et al., 2007). Werden beide Populationen infiziert, kommt es zu bidirektionaler CI. Bei Infektion von nur einer Population tritt unidirektionale CI als Isolationsmechanismus in Erscheinung, wenn der Kontakt zwischen den Subpopulationen wiederhergestellt wird. Die betreffenden Studien ignorieren aber, dass die Subpopulationen sehr wahrscheinlich auch genetischen Veränderungen unterlaufen sind und dass daher neben CI auch nukleare Inkompatibilitäten auftreten sollten. Insbesondere Haldansche, geschlechtsspezifische Inkompatibilitäten wurden häufig in Schmetterlingen und Fruchtfliegen (*Drosophila*) beobachtet (Coyne, 1992), und in beiden Gruppen wurden zahlreiche *Wolbachia*-Infektionen nachgewiesen. Ein gemeinsames Auftreten von CI und NI ist daher sehr wahrscheinlich.
Schließlich ist die allgemeine Rolle von *Wolbachia* in Evolutionsprozessen von der Anzahl infizierter Arten abhängig. Aktuelle Schätzungen implizieren dass circa 20% aller Insekten mit *Wolbachien* infiziert sind (Werren et al., 1995a, Werren and Windsor, 2000). Aufgrund der Datenerhebungsmethoden ist aber offensichtlich, dass es sich dabei um Unterschätzungen handelt und wahrscheinlich viel mehr Arten infiziert sind. In dieser Arbeit werden wir dieses Problem diskutieren und vorhandene Daten im Rahmen eines statistischen Modells analysieren.

Methoden und Ergebnisse: Artbildung

In dieser Arbeit soll das Zusammenspiel von zytoplasmatischen und nuklearen Inkompatibilitäten theoretisch untersucht werden. Wir nutzen das klassische Dobzhansky-Muller Modell und erweitern es um *Wolbachia*-Infektionen.

Wir analysieren den wechselseitigen Einfluss von nuklearer und zytoplasmatischer Inkompatibilität bezüglich der Stabilität der Isolationsmechanismus. Die Stabilität wird in Form der sogenannten kritischen Migrationsrate gemessen. Diese ist definiert als höchste Migrationsrate zwischen den Subpopulationen die die Erhaltung der genetischen bzw. zytoplasmatischen Divergenz erlaubt. Wird die kritische Migrationsrate überschritten, geht diese Divergenz und somit der jeweilige Isolationsmechanismus verloren.
Im klassischen Dobzhansky-Muller Modell analysieren wir Interaktionen für einen großen Parameterraum. Dabei unterscheiden wir zwischen rezessiven und dominanten nuklearen Inkompatibilitäten und untersuchen den Einfluss von unidirektionaler, asymmetrischer und symmetrischer bidirektionaler CI. Als Hauptergebnisse dieser Analyse erhalten wir zum einen, dass bidirektionale CI als starker Stabilisator für genetische Divergenz wirkt. Dies ist insbesondere wichtig für rezessive NI, da diese ohne *Wolbachia*-Infektionen leicht verloren gehen, aber im allgemeinen häufiger auftritt als dominante NI. Zum anderen kann auch unidirektionale CI genetische Divergenz stärken, allerdings unter weniger allgemeinen Bedingungen. Im Gegenzug können die nuklearen Inkompatibilitäten den Infektionspolymorphismus von infizierten und nichtinfizierten Individuen stabilisieren. In einigen Fällen kann es sogar Synergyeffekte geben, d.h. beide Mechanismen verstärken sich gegenseitig so, dass sie gemeinsam auftretend weitaus stabiler sind als beim alleinigen Auftreten.
Weiterhin untersuchen wir Interaktionen zwischen geschlechtsspezifischer Haldanscher NI und unidirektionaler CI. Hierbei ist interessant, dass abhängig von der Geschlechtsbestimmung verschiedene Effekte auftreten. Haldansche NI betrifft immer das heterogametische Geschlecht. Die Geschlechtsbestimmung in *Drosophila* zum Beispiel erfolgt über XY-Geschlechtschromosomen. Männchen sind heterogametisch (XY) und daher von Haldanscher NI betroffen. Lepidopteren, also Motten und Schmetterlinge, haben ZW-Geschlechtschromosomen. Männchen sind homogametisch (ZZ) und Weibchen heterogametisch (ZW) und entsprechend zeigen letztere Haldansche Sterilität oder Lethalität. Unsere Ergebnisse zeigen dass Haldansche NI in Lepidopteren als effektive Barriere gegen die Ausbreitung einer *Wolbachia*-Infektion wirkt. Da nur Frauen *Wolbachien* auf die nächste Wirtsgeneration übertragen können, wird durch die Lethalität infizierter Frauen die Ausbreitung der Bakterien verhindert. Das führt zu einer erhöhten Stabilität von CI als Isolationsmechanismus, der dann seinerseits NI stabilisieren kann. Dieser Synergieffekt konnte für XY-Arten nicht festgestellt werden. Hier verursacht NI Lethalität von infizierten Männchen, was die Ausbreitung von *Wolbachia* nicht effektiv verhindern kann.
Insgesamt zeigen die Ergebnisse, dass *Wolbachia*-induzierte CI einen star-

ken und allgemeinen Einfluss auf klassische Artbildungsmechanismen haben kann. Bidirektionale CI verstärkt genetische Inkompatibilitäten signifikant. Auch unidirektionale CI stabilisiert NI, allerdings unter eingeschränkteren Bedingungen. Speziell in Lepidopteren können aber starke Effekte auftreten, nämlich wenn Haldansche NI die Ausbreitung der Bakterien verhindert.

Methoden und Ergebnisse: Wie viele Arten sind infiziert?

Die eigentliche Bedeutung der *Wolbachien* in der Artbildungstheorie wird natürlich auch von der Häufigkeit ihres Auftretens abhängen. Existierende Studien schätzen, dass *Wolbachien* circa 20% aller Insektenarten infizieren (Werren et al., 1995a, West et al., 1998). Betreffende Studien sammeln dazu zahlreiche Individuen verschiedener Arten und testen auf Präsenz von *Wolbachia*. Dabei wird häufig nur ein Individuum pro Art getestet. Ist dieses infiziert, wird die Art als infiziert klassifiziert. Ist es nicht infiziert, gilt die gesamte Art als nichtinfiziert. Diese Methode greift, wenn *Wolbachien* immer jedes einzelne Individuum infizieren. Es wurden allerdings auch niedrige Infektionsdichten von 10% und weniger gefunden. Diese Infektionen wären vermutlich nicht mit dem Testen eines einzelnen Individuums entdeckt worden. Daher vermuteten einige Autoren (Werren et al., 1995a, Tagami and Miura, 2004), dass in Wirklichkeit viel mehr Arten als die bislang angegebenen 20% infiziert sind. In dieser Arbeit präsentieren wir die erste statistische Metaanalyse von veröffentlichten Daten zu Infektionsraten von *Wolbachia*. Wir analysieren den Datensatz im Rahmen eines Betabinomial-Modells. Dabei schätzen wir eine Funktion, die die Verteilung der Infektionsdichten innerhalb der Arten beschreibt. Basierend auf dieser Funktion erhalten wir Schätzer für die Gesamtzahl der infizierten Arten. Die Funktion zeigt eine U-förmige Verteilung, d.h. *Wolbachia*-Infektionen treten entweder in sehr niedrigen (<10%) oder sehr hohen (>90%) Frequenzen innerhalb einer Art auf. Desweiteren impliziert unsere Analyse, dass ungefähr zwei Drittel aller getesteten Arten mit *Wolbachien* infiziert sind.

Konklusion

Insgesamt liefert diese Arbeit viele Indizien dafür, dass *Wolbachien* eine allgemeine und einflussreiche Rolle in Artbildungsprozessen von Arthropoden spielen. Zum einen haben wir gezeigt, dass *Wolbachia*-induzierte zytoplasmatische Inkompatibiltät genetisch-basierende Artbildungsfaktoren begünstigt. Insbesondere konnten wir zeigen, dass dieses nicht nur für bidirektionale CI, sondern ebenfalls für vermutlich sehr viel häufiger auftretende unidirektionale CI der Fall ist. Speziell in Lepidopteren kann unidirektionale CI im Zusammenspiel mit Haldanscher NI starke artbildungsfördernde Effekte auslösen. Daraus folgt, dass zytoplasmatische Inkompatibilität als allgemeiner Artbildungsmechanismus anerkannt werden kann. Zum anderen haben wir gezeigt, dass mit bis zu 70% weitaus mehr Arten mit *Wolbachia* infiziert sind

als die bislang angenommenen 20%. Sowohl unsere Untersuchungen zur klassischen Artbildungstheorie als auch die statistische Analyse von Infektionsdaten suggerieren dass *Wolbachien* allgemein als Artbildungskatalysatoren in Arthropoden anerkannt werden sollten.

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Selbständigkeitserklärung

Hiermit erkläre ich, dass ich die Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Berlin, den 15. Dezember 2008

Kirsten Hilgenböcker